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Proteomic profiling of individual bovine cumulus complexes after *in vivo* and *in vitro* maturation reveals aberrations in numerous biological pathways

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Table of Contents

1 Zusammenfassung

Die Kumuluszellen umgeben ihre Eizelle während der gesamten präovulatorischen Entwicklung. Dabei stellen sie nicht nur einen mechanischen Schutz dar, sondern sie kommunizieren mit der Eizelle durch den bidirektionalen Austausch von Metaboliten. Die Kumuluszellen stellen einen Spiegel des Oozyten-Zustandes dar und sind damit eine interessante Quelle um die Entwicklungskompetenz der Oozyte non-invasiv untersuchen zu können.

Die *in vitro* Maturation stellt einen ersten limitierenden Schritt in der *in vitro* Embryoproduktion dar. Die Analyse der Proteinexpression von Kumuluszellen während dieser kritischen Phase kann helfen den Einfluss der Maturationsbedingungen auf die Eizellqualität zu untersuchen. Das Ziel dieser Studie war es daher, das Kumulusproteom von Eizellen die erfolgreich maturierten oder nicht maturierten unter *in vivo* und *in vitro* Maturationskonditionen zu untersuchen.

Es wurden 20 Kumuluskomplexe von einzelnen Oozyten analysiert. Die Hälfte wurde *in vivo* und die andere Hälfte *in vitro* maturiert. Für die beiden Maturationsgruppen wurde Kumulus von erfolgreich maturierten Oozyten (n=5) und Oozyten die nicht erfolgreich maturierten (n=5) gewonnen. Oozyten Spender der Studie waren sechs Brown Swiss Färsen ähnlichen Alters, body condition scores und aufgezogen unter ähnlichen Bedingungen (n=3 *in vivo* und *in vitro*). Für die Gewinnung *in vivo* maturierter Kumulus-Oozyten-Komplexe (KOK) wurden die Färsen Östrus synchronisiert und superovuliert. Sie wurden 24 Stunden nach Ovulationsinduktion geschlachtet. Die KOK der *in vitro* Gruppe wurden unter Progesteronbehandlung (Tag 5) gewonnen und in Einzelkultur für 21 Stunden *in vitro* maturiert. Zur Probengewinnung wurde der Kumulus von den gereiften Oozyten entfernt, in PBS gewaschen und in Flüssigstickstoff gefroren und bis zur Analyse gelagert. Die korrespondierenden Oozyten wurden auf Polkörperchen Ausscheidung untersucht, um eine erfolgreiche Maturation festzustellen.

Die Analyse der Kumuluskomplexe erfolgte mittels Massenspektrometrie. Für die Zelllyse und den Proteinverdau wurde ein Filter assistiertes Präparationsprotokoll angewendet. Die Daten wurden mit Hilfe der ProgenesisQI Software (NonlinearDynamics) quantifiziert. Die vier biologischen Gruppen wurden paarweisen Analysen unterzogen. Für einen signifikanten Unterschied wurden die Grenzen bei einem zweifachen Anstieg der Proteinexpression zusammen mit einem p-Wert < 0.05 (t-Test) gesetzt.

Insgesamt wurden 2277 quantifizierbare Proteine in den 20 Proben identifiziert. Zwischen den erfolgreich maturierten KOK nach *in vivo* und *in vitro* Maturation waren 459 Proteine signifikant unterschiedlich exprimiert (308 Proteine überexprimiert in der *in vivo* Gruppe und 151 Proteine überexprimiert in der *in vitro* Gruppe). Im Vergleich der Gruppen die nach *in vivo* und *in vitro* Maturation nicht erfolgreich maturierten waren 152 Proteine signifikant unterschiedlich exprimiert (63 Proteine überexprimiert in der *in vivo* maturierten Gruppe und 89 Proteine in der *in vitro* maturierten Gruppe). Zwischen den *in vivo* maturierten Oozyten die erfolgreich maturierten oder nicht maturierten wurden 360 signifikant unterschiedliche Proteine identifiziert (240 Proteine überexprimiert in der erfolgreich maturierten Gruppe und 120 Proteine in der Gruppe der nicht maturierten KOK). Hingegen waren im Vergleich der *in vitro* maturierten Gruppen nur 19 Proteine signifikant unterschiedlich exprimiert (13 Proteine überexprimiert in der erfolgreich maturierten Gruppe und 6 Proteine in der Gruppe die nicht erfolgreich maturierte). Eine Anreicherungsanalyse mit Hilfe der STRING-Datenbank ergab überrepräsentierte KEGG Stoffwechselpfade in zwei der Gruppenvergleiche. Im Vergleich der erfolgreich maturierten KOK waren nach *in vivo* Maturation 4 Stoffwechselpfade im Vergleich zur *in vitro* Maturation überrepräsentiert: Komplement- und Koagulationskaskade (21 Proteine; $p<0.0001$), Steroid Biosynthese (7 Proteine, $p=0.0025$), N-Glykan Biosynthese (7 Proteine, $p=0.04$) und ECM-Rezeptor Interaktion (11 Proteine, $p=0.04$). Beim Vergleich der *in vivo* maturierten Gruppen waren 3 Stoffwechselpfade in den KOK die erfolgreich maturierten signifikant überrepräsentiert: Komplement- und Koagulationskaskade (21 Proteine, $p<0.0001$), ECM-Rezeptor Interaktion (11 Proteine, $p=0.01$) und Ovarielle Steroidogenesis (5 Proteine, $p=0.058$).

Neben diesen überrepräsentierten KEGG Stoffwechselpfaden gab es diverse Proteine die weiteren biologischen Funktionen von besonderem Interesse im *Cumulus oophorus* der Oozyte zugeordnet werden konnten. Diese wurden für die Diskussion den Gruppen Abwehr des Oxidativen Stresses, Modulation der Apoptose, Reparatur von DNA Schädigung, Gas Transport, Stabilität und Expansion des Kumulus, Post ovulatorische Prozesse und Spermien Beeinflussung zugeteilt.

Die Studie präsentiert ein neues, hochsensitives analytisches Werkzeug das die Analyse der minimalen Zellmengen von einzelnen Kumuluskomplexen erlaubt. Die Veränderungen im Proteom zwischen den unterschiedlichen Maturationsbedingungen, aber auch zwischen KOK mit und ohne Maturationskompetenz waren enorm. Es wurden diverse biologische Prozesse und Stoffwechselpfade identifiziert, die eine bedeutende Rolle für die Entwicklungskompetenz der KOK spielen.

2 Summary

The oocyte forms a complex with their somatic cumulus cells within the follicle throughout the preovulatory maturation steps. Cumulus cells support their oocyte not only through mechanical protection but also with a close bidirectional exchange of metabolites. Analysis of the oocytes cumulus gives the opportunity to explore non-invasively oocytal well-being and quality.

In vitro maturation (IVM) is the first rate-limiting step in *in vitro* embryo production. Analysis of protein expression in cumulus cells around this critical step helps to explore the impact of maturation conditions and to examine an influence on maturational competence of the oocyte. The goal of this study was the comparison of the cumulus proteome of oocytes with and without maturational competence matured under *in vivo* and *in vitro* conditions.

Therefore twenty cumulus samples corresponding to single oocytes were analysed. Half of the samples were matured *in vivo* and the other half *in vitro*. For each maturation group, cumulus from oocytes matured successfully (n=5) and failed to mature (n=5) were analysed.

Six Brown Swiss heifers of similar age, body condition score and raising conditions were used for this study (n=3 for each maturation condition). For cumulus-oocytes complexes (COCs) matured under *in vivo* conditions heifers were oestrus synchronised, eCG superovulated and slaughtered 24 hours after final GnRH injection and progesterone withdrawal. COCs for the *in vitro* group were collected after oestrous synchronisation on day 5 of progesterone treatment and matured in single culture for 21 hours. Cumulus samples were removed from their oocyte, washed in PBS, snap frozen and stored in liquid nitrogen. Extrusion of first polar body was used to confirm the maturation success and classify the COCs in successfully matured and failed to mature.

Cumulus analysis was performed by a mass spectrometry (MS) based protein profiling approach. For cell lysis and protein digestion an adapted filter-aided sample preparation protocol was used. Data were analysed by label-free quantification using ProgenesisQI software (NonlinearDynamics). The four biological groups underwent pairwise comparisons. For significant differences a fold change in protein expression of >2 along with $p<0.05$ (t-Test) was assumed.

A total of 2277 quantifiable proteins were identified in the 20 single COC samples. Between the successfully matured COCs that underwent *in vivo* or *in vitro* maturation 459 proteins were differently expressed (308 proteins with the highest mean in the *in vivo* matured group; 151 proteins with the highest mean in the *in vitro* matured group). For the groups that failed to

mature after *in vivo* or *in vitro* maturation 152 proteins were significantly different expressed (63 proteins with the highest mean in the *in vivo* matured group; 89 proteins with the highest mean in the *in vitro* matured group). Comparison of *in vivo* matured COCs that matured successfully or failed to mature revealed 360 proteins with significantly different expression (240 proteins with highest mean in successfully matured COCs; 120 proteins with highest mean in COCs that failed to mature). Between the *in vitro* matured COCs only 19 proteins were significantly different expressed (13 proteins with highest mean in successfully matured COCs; 6 proteins with highest mean in COCs that failed to mature). Enrichment analysis using the String-Database revealed overrepresentation of KEGG pathways for two groups comparisons. In successfully matured COCs four pathways were overrepresented after *in vivo* maturation compared to *in vitro* maturation: Complement and coagulation cascades (21 proteins, $p<0.0001$), Steroid biosynthesis (7 proteins, $p=0.0025$), N-Glycan biosynthesis (7 proteins, $p=0.04$) and ECM-receptor interaction (11 proteins, $p=0.04$). For the *in vivo* matured groups three pathways were overrepresented in cumulus of successfully matured oocytes compared to cumulus of oocytes that failed to mature: Complement and coagulation cascades (21 proteins, $p<0.0001$) and ECM-receptor interaction (11 proteins, $p=0.01$), Ovarian steroidogenesis (5 proteins, $p=0.058$).

Beneath these overrepresented pathways individual significantly different expressed proteins that can be assigned to the following biological functions of special interest in the cumulus oophorus were chosen for discussion: Oxidative stress defence, modulation of apoptosis, repair of DNA damage, gas transport, stability and expansion of cumulus, post ovulatory processes and influence on sperm.

This study presents a novel, highly sensitive tool that allowed the proteomics analysis of minute sample amounts of cumulus complexes corresponding to single oocytes.

The results revealed major aberrations in the cumulus proteome of oocytes with and without competence to mature and between the two maturation conditions. Several pathways and biological functions were identified that might be responsible for the maturational competence of the oocytes.

3 Introduction

During the critical step of maturation a rich bidirectional exchange of metabolites occurs between oocytes and their neighbouring somatic cells of the cumulus complex (CC). The CC provides a favourable microenvironment that is necessary for oocyte growth and development. These nursing cells transfer metabolic substrates, eliminate disturbing substances and modulate environmental influences (Cetica et al., 1999b; Hashimoto et al., 1998).

Development potential of oocytes was described as reduced after maturation *in vitro* compared to oocytes matured under *in vivo* conditions. Metabolism within the COC is altered and gene expression in oocytes as well as cumulus cells is different (Lonergan, 2013). Several studies suggest that gene expression in cumulus cells could be able to predict oocyte development competence (Bunel et al., 2013). Studies on the cumulus proteome are only scarce, even though the proteome corresponds more to the metabolic phenotype of cells than the transcriptome (Anderson and Anderson, 1998).

The availability of novel highly sensitive mass spectrometry based protein analysis methods raised the hypothesis that characterisation of protein expression in cumulus complexes might be possible even on single oocyte level (Wisniewski et al., 2009). This would provide the unique opportunity to relate the cumulus proteome to the maturational and developmental competence of the corresponding oocyte. Therefore the goal of this study, beneath a first characterisation of protein expression in cumulus cells on single oocyte level, was the analysis of aberrations in the cumulus proteome between different maturation outcome and condition. The comparison of protein expression after *in vivo* and *in vitro* maturation may contribute to a better comprehension of limitations of maturation *ex vivo*. The correlation of the cumulus proteome to the maturation stage of the corresponding oocyte might reveal potential biomarkers to predict the oocytes maturational competence.

In summary, the results of this study may contribute to optimization of *in vitro* embryo production by elucidation of limitations in the *in vitro* maturation conditions. Identification of biomarkers for maturational competence of the corresponding oocyte may provide novel selection criteria for oocytes in the future.

4 Literature

4.1 Metabolism of the Cumulus-Oocyte-Complex during maturation

4.1.1 Maturation of oocytes

Oocytes arrest their maturation during foetal life in late prophase of first meiotic division. After the surge of luteinising hormone (LH), during estrus and short before ovulation, meiosis resumes and the oocyte reaches metaphase II (MII). To acquire the competence for fertilisation the oocyte has to undergo this final maturation. The successfully matured oocyte extruded their first polar body and arrests again in MII until fertilisation (Hyttel et al., 1986). The complex process of maturation takes place in the preovulatory follicle. For *in vitro* embryo production, oocytes are usually collected from antral follicles by aspiration and matured under artificial conditions mimicking the physiological surrounding. The maturation process results in changes in a variety of cellular components: the plasma membrane, the nucleus as well as the cytoplasm (Brackett, 1985).

4.1.2 Cumulus-oocyte-complex: Anatomy and Communication

The cumulus oophorus (CO) consists of several layers of somatic cells (cumulus cells) surrounding the oocyte from antral follicle-stage up to fertilisation. Originating from granulosa cells, the cells closest to the oocyte will differentiate into cumulus cells and cells with greater distance to the oocyte into mural granulosa cells (Eppig et al., 1997). This differentiation occurs through formation of the antrum during follicular development and is influenced by the oocyte (Emori and Sugiura, 2014; Eppig et al., 1997). Cumulus and mural granulosa cells present after that a different phenotype in anatomy and functions (Emori and Sugiura, 2014; Eppig et al., 1997; Li et al., 2000).

The initially tight cumulus, characteristic for immature oocytes, undergoes during the maturation process an expansion, which is also called activation or mucification (Brackett, 1985). Beside an increased number of cumulus cells during the process (Cetica et al., 2001), hyaluronic acid production in the extracellular matrix between cumulus cells is responsible for this expansion (Brackett, 1985).

The *cumulus oophorus* plays a crucial role in successful maturation (Shimada, 2013) and acquisition of developmental competence of oocytes (Atef et al., 2005).

Cumulus cells provide an optimal microenvironment to supply the oocyte with nutrients and eliminate inhibitory or toxic components (Atef et al., 2005; Hashimoto et al., 1998).

Cumulus cells and their respective oocyte communicate through gap junctions during growth and maturation of the gamete. The first layer of cumulus cells, the corona radiata, releases cytoplasmatic projections through the zona pellucida of the oocyte (transzonal processes) (de Loos et al., 1991). Small molecular weight products (glucose metabolites, amino acids, nucleotides, small regulatory molecules like cAMP, purines or transcripts (Macaulay et al., 2016; Sutton et al., 2003b)) pass through gap junctions at the end of the transzonal processes permitting juxtacrine communication (de Loos et al., 1991).
Gap-junctional communication seems to be necessary and remains at least partially present up to the end of maturation to permit normal bovine oocyte maturation (Vozzi et al., 2001). Physical contact between oocyte and cumulus cells is necessary up to fertilization (Fatehi et al., 2005; Tanghe et al., 2003). Roles in egg-sperm interaction, spermatozoa attraction toward oocyte, induction of capacitation, influence on sperm motility and penetration were reviewed (Fatehi et al., 2005). The removal of cumulus cells before IVF leads to decreased developmental competence of oocytes (Macaulay et al., 2016; Tanghe et al., 2002), even when free cumulus cells are added to the maturation and/or fertilisation medium (Fatehi et al., 2002; Zhang et al., 1995). Maturation up to MII is described in absence of direct oocyte-cumulus cell communication like in denuded oocytes (Zhang et al., 1995) but with impaired oocyte quality and reduced further development potential. A similar detrimental effect on oocytes developmental competence is observed when intact COCs are matured in presence of gap junction inhibitors (Atef et al., 2005).
Another type of communication beside this juxtacrine way is a paracrine communication with signal molecules (oocyte-secreted factors) that diffuse between gametes and cumulus and play a major role in determination of granulosa cell differentiation (Eppig et al., 1997). These juxtacrine and paracrine exchanges are essential for oocyte growth, maintenance of meiotic arrest and resumption, maturation and preparation of a qualitative good gamete (Eppig, 1991; Hussein et al., 2006; Thomas et al., 2004).

Impressive examples of the “cumulus to oocyte” communication are the ways in which meiotic arrest is maintained (Seli et al., 2014) and how LH indirectly induces maturation of the oocyte. In absence of LH-receptors on the oocyte and only poor expression on cumulus cells (Peng et al., 1991; Shimada, 2013), granulosa cells are able to mediate the meiotic

resumption of oocytes via cumulus cells. LH acts on mural granulosa cells to reduce inhibitors of meiotic resumption that maintained the meiotic arrest via cumulus cells (Thomas et al., 2004). Well described in rodents (Aktas et al., 1995; Liu et al., 2013; Norris et al., 2008; Norris et al., 2009), this process is not completely understood in the bovine species, where it seems to be more complex, involving several signalling pathways (Bilodeau-Goeseels, 2011). Farmer (2014) provides a detailed description on the regulation of oocyte meiotic resumption by cAMP modulators in bovine *in vitro* maturation.

The importance of cumulus cells in regulation of oocyte maturation in pig oocytes was already described in 1979 by Hillensjo and coworkers: an oocyte maturation inhibitor is present in follicular fluid and responsible for the meiotic arrest of oocytes. It needs the presence of cumulus cells to control the maturation of oocytes and also influences the differentiation of cumulus cells (Hillensjo et al., 1979).

Parallel to the influence of cumulus cells on oocytes, the oocyte regulates via oocyte-secreted factors (OSFs) the cumulus cells phenotype and therefore its own microenvironment (Gilchrist et al., 2004; Gilchrist and Thompson, 2007).

Numerous influences of oocyte-secreted factors on the surrounding somatic cells in mammals were reviewed by Australian authors (Gilchrist et al., 2004; Thompson, 2013). Effects of OSFs were described on:

- Development of the follicle / regulation of oocyte development / differentiation, function and proliferation of cumulus cells and mural granulosa cells (Diaz et al., 2007b; Emori and Sugiura, 2014; Eppig et al., 1997; Eppig et al., 2002; Gilchrist et al., 2001; Gilchrist et al., 2006; Glister et al., 2003; Joyce et al., 1999; Lanuza et al., 1998; Li et al., 2000; Su et al., 2003; Vanderhyden et al., 1992)
- Metabolism of cumulus cells with stimulation of glycolysis, cholesterol synthesis, amino acid uptake in cumulus cells, stimulation of transfer of cAMP and energy substrates to oocyte (Eppig et al., 2005; Su et al., 2008; Sugimura et al., 2014; Sugiura et al., 2005)
- Expansion of cumulus (Buccione et al., 1990; Ralph et al., 1995; Singh et al., 1993)
- Prevention of cumulus cells luteinisation and modulation of steroid production (Glister et al., 2003; Li et al., 2000)
- Prevention of cumulus apoptosis (Hussein et al., 2005)
- Oocyte quality (Hussein et al., 2011; Hussein et al., 2006)

During maturation, rich exchanges of metabolites are observed between both cell types. Studies showed that metabolic profiles of denuded bovine oocytes or oocytectomised complexes differed from the ones where complete COCs were matured (Khurana and Niemann, 2000; Zuelke and Brackett, 1992, 1993). For the examination of metabolism during maturation, the COC has then to be considered as a whole unit (Krisher, 2013; Sutton et al., 2003b). The metabolism during maturation and metabolic rate within the COC, of oocyte as well as cumulus cells, is known being associable with further oocyte quality (Thompson et al., 2007).

The metabolism in the COC provides energy, material, controls of stops and starts in meiotic progression as well as regulation of oxidative stress (Krisher, 2013). The rapid changes in maturing COCs implies high needs in substrates (Sutton et al., 2003a). To cover energetic and anabolic needs, oocytes use different metabolites: lipids, amino acids and glucose (Collado-Fernandez et al., 2012; Sutton et al., 2003b).

During the growth phase of the oocyte, before onset of maturation, stores of nutrients (like glycogen granules and lipid droplets, mRNA and proteins) or organelles are produced to prepare the oocyte for the further developments (Collado-Fernandez et al., 2012; Dunning et al., 2014). Changes like post-transcriptional modifications of mRNAs, protein synthesis, post-translational regulation and reorganisation of organelles happens then during the cytoplasmic maturation (Collado-Fernandez et al., 2012).

The metabolism is influenced by maturation conditions as oocytes are nursed by their direct microenvironment: the cumulus oophorus and the maturation medium *in vitro* or the follicular fluid *in vivo* (Dunning et al., 2014). The follicular fluid composition correlates with serum biochemical profile and is influenced by several factors like maternal energy balance, milk production, nutrition or heat stress (Alves et al., 2014; Alves et al., 2013; Leroy et al., 2008; Leroy et al., 2004). Variations of such factors influence the reproductive efficiency: one factor for this reduction of fertility is a possible failure of maturation of the oocyte due to metabolic disturbances (De Wit et al., 2001; Hashimoto et al., 2000b; Leroy et al., 2005).

Several metabolic pathways are involved during the maturation process with different distribution in both cell types. The consumption of metabolites is also variable between the oocyte and the cumulus cells. As example, cumulus cells have a higher demand for glucose and lower need for oxygen and oxidative substrates in comparison with the oocyte

(Thompson et al., 2007). The following sections will discuss the different metabolic pathways important for COC maturation.

4.1.3 Carbohydrate metabolism

The most important carbohydrate during maturation of the COC is glucose, its metabolism is the best examined and was reviewed by Sutton-McDowall et al. in 2010 (Sutton-McDowall et al., 2010). Beside a precursor for energy production, glucose provides constitutive, regulatory and structural material essential for successful maturation. When only the needs for energy production are provided to denuded oocytes, they still aren't able to finish maturation, as the other roles of glucose are necessary to complete this stage with success (Sutton-McDowall et al., 2010).

Cumulus cells are necessary for successful glucose valorisation in the COC (Seli et al., 2014) with their high potential to change it in usable substrates for the oocyte (Songsasen, 2012). Bovine oocytes possess a low capacity to use it on its own due to its limited amount of glucose transporters (Songsasen, 2012).

Different pathways of glucose metabolism in the COC are described in the literature (Songsasen, 2012; Sutton-McDowall et al., 2010) (Figure 1):

- Glycolysis
- Pentose phosphate pathway (PPP)
- Hexosamine biosynthesis pathway (HBP)
- Polyol pathway

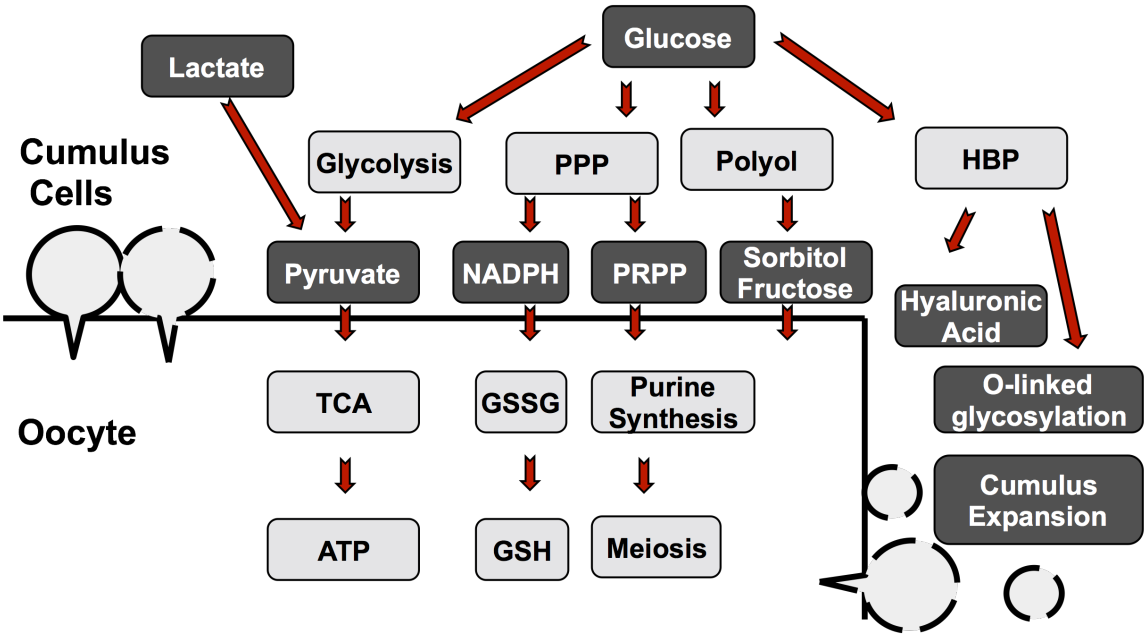

Figure 1: Schema of the four pathways of glucose metabolism within the COC, adapted from Sutton-McDowall et al. (2010). Glycolysis produce pyruvate and oocytes use pyruvate for production of energy (ATP), through tricarboxylic acid (TCA) cycle and oxidative phosphorylation. Pentose phosphate pathway (PPP) produces NADPH, which is involved in antioxidant defence by reduction of glutathione (GSSG to GSH) and also transforms glucose into precursors of purine nucleotides (phosphoribosylpyrophosphate, PRPP) for regulation of meiosis. Polyol pathway (polyol) provides sorbitol and fructose by oxidation of glucose. Hexosamine biosynthetic pathway (HBP) permits glucose to participate in hyaluronic acid synthesis for cumulus expansion and, through O-linked glycosylation of proteins, in cell signalling.

Glycolysis in the COC represents the main pathway for energy production in form of adenosine triphosphate (ATP), pyruvate and lactate (Sutton-McDowall et al., 2010). Glycolysis plays beside this an important role in redox regulation (Sutton-McDowall et al., 2010).

Cumulus cells and their oocyte interfere to optimize this metabolic way. Cumulus cells, with a most effective glucose transporter (Dan-Goor et al., 1997) and a better enzymatic activity (Cetica et al., 2002), convert the glucose in metabolites the oocyte is able to consume. Glycolysis is for cumulus cells the main way to metabolise glucose (Cetica et al., 2002). Glycolysis in cumulus cells is regulated by oocytes through OSFs (Sugiura et al., 2007).

Oocytes then gain energy through transport of metabolites like pyruvate from its cumulus or oxidation of extracellular pyruvate (Leese and Barton, 1984; Rieger and Loskutoff, 1994).
Oocytes use pyruvate for production of ATP, through tricarboxylic acid (TCA) cycle and oxidative phosphorylation (Dumollard et al., 2009; Dumollard et al., 2007; Rieger and Loskutoff, 1994).
Pyruvate can also be gained, in both cell types, by oxidation of lactate by cytosolic lactate dehydrogenase (LDH), with a role in regulation of cytosolic redox state (Cetica et al., 1999b; Dumollard et al., 2009; Dumollard et al., 2007).
The signal for onset of maturation, LH, impacts COC glucose metabolism by promoting glycolysis and TCA cycle (Zuelke and Brackett, 1992).

The pentose phosphate pathway (PPP) transforms glucose into precursors of purine nucleotides (phosphoribosylpyrophosphate, PRPP), for biosynthetic pathways like DNA/RNA synthesis and control/resumption of nuclear maturation. Another important role of pentose phosphate pathway is reduction of nicotinamide adenine dinucleotide phosphate (NADP+) in nicotine adenine dinucleotide phosphate hydrogen (NADPH), which is involved in antioxidant defence by reduction of glutathione (GSSG to GSH) (Collado-Fernandez et al., 2012; Gutnisky et al., 2014; Sutton-McDowall et al., 2010). PPP occurs mostly in oocytes, beside a small activity in cumulus cells (Cetica et al., 2002).

In hexosamine biosynthesis pathway (HBP), glucose and glutamine take part to hyaluronic acid synthesis for cumulus expansion and, through O-linked glycosylation of proteins, in cell signalling (Collado-Fernandez et al., 2012; Sutton-McDowall et al., 2010). According to its functions, this glucose metabolising pathway increases towards the end of maturation (Sutton-McDowall et al., 2004). The HBP takes place in both cell types (Sutton-McDowall et al., 2006; Thompson, 2006), with a majority of activity in cumulus (Thompson, 2006).

The polyol pathway provides sorbitol and fructose by oxidation of glucose in granulosa cells and oocytes. This pathway remains low under normal glycaemic conditions and increases by hyperglycaemia with negative impact on oocyte quality (Collado-Fernandez et al., 2012; Sutton-McDowall et al., 2010).

These different pathways transform glucose to provide secondary metabolites to the entire COC. Substrates are used intracellular by the oocyte for energy production, nuclear

maturation, progression of meiosis regulation and redox state (Sutton-McDowall et al., 2010). Substrates are also needed for extracellular processes like cumulus expansion and cell signalling (Sutton-McDowall et al., 2010). Tight regulation of the pathways is necessary to mature a good quality bovine oocyte. Inhibition or stimulation of glucose metabolism is directly associated to maturation success and developmental competence of bovine oocytes (Hashimoto et al., 2000b; Krisher and Bavister, 1999; Steeves and Gardner, 1999; Sutton-McDowall et al., 2006).

Dysbalances, as consequence of a too high or too low glucose concentration during maturation, show repercussions as disturbed cytoplasmic maturation, impaired completion of maturation, abnormal mucification and reduced development potential (Sutton-McDowall et al., 2010).

4.1.4 Protein and nucleic acids. Amino acid metabolism

Maturation is a highly active period of protein synthesis. Variations of the pattern of protein synthesis can be observed within the bovine COC along IVM (Coenen et al., 2004). Depending of its function, a protein is produced at a specific moment of maturation, specifically in the oocyte or in the cumulus cells (Wu et al., 1996).

Beside the role of amino acids as substrates for this *de novo* protein synthesis, other functions in the oocyte were reviewed by Collado-Fernandez and coworkers: Amino acids are involved in energy production, in several synthesis processes (nucleotides, glycoproteins, hyaluronic acid, signalling molecules) and participate in regulation of pH and osmolarity (Collado-Fernandez et al., 2012).

A tight communication between cumulus cells and oocytes, via gap-junctions and paracrine, is responsible for optimisation of amino acid metabolism (Seli et al., 2014). The mammalian cumulus cells possess much larger amounts of some essential enzymes for amino acid metabolism and transporters than oocytes (Cetica et al., 2003; Seli et al., 2014). Cumulus cells can collect amino acids from their microenvironment and transport it into the oocyte via gap junctions (Seli et al., 2014). Formation of amino acid uptake transporters on cumulus cells is promoted by the oocyte via paracrine factors (Eppig et al., 2005).

Cumulus cells also modulate the influence of gonadotropins like LH on amino acid and glucose metabolism in cumulus-enclosed oocyte (Zuelke and Brackett, 1992, 1993).

In oocytes, depletion in medium of glutamine, arginine, asparagine as well as the production of alanine can be observed during IVM (Hemmings et al., 2012). Further oocyte competence is correlated with the amino acid profile (Hemmings et al., 2012) as well as with the amino acid turnover. The developmental potential is decreasing with higher turnover rate (Hemmings et al., 2012). Glutamine supplementation to IVM media improves completion of maturation in cattle oocytes significantly (Bilodeau-Goeseels, 2006). As metabolic precursor of the HBP, glutamine in the medium influences cumulus expansion through production of hyaluronic acid (Furnus et al., 1998). Glutamine, like glycine, is also involved in energy production through metabolization in the TCA cycle; LH increases the turnover rate in bovine oocyte with positive impact on oocyte quality (Rieger and Loskutoff, 1994; Seli et al., 2014; Zuelke and Brackett, 1993). Glutamine participates also in Glutathione (GSH) composition. GSH is a glutamine-cysteine-glycine tripeptide which synthesis is increased during maturation (Zuelke et al., 2003). It accumulates in oocytes and plays a role in oxidative stress protection later in oocyte life (Cetica et al., 2001; Furnus et al., 2008; Luberda, 2005). Cumulus cells contribute to GSH oocyte store by providing substrates for synthesis or directly transferring GSH to the oocyte (de Matos et al., 1997; Mori et al., 2000). Cumulus cells are able to change cystine in cysteine (Sawai et al., 1998). Cystine, as unstable essential amino acid, represents a limiting factor for GSH production (Furnus et al., 2008). Cysteine it then transferred to the oocyte as a constitutive amino acid of GSH together with glycine and glutamine (de Matos et al., 1997; Furnus et al., 2008). Supplementation of cysteine in IMV medium is described to increase GSH concentration and competence in the bovine oocytes (Hidaka, 2018).

These different examples image implication of amino acids in successful oocyte maturation. The amino acids are necessary to this process and their needs vary in a stage-dependent manner during the process (Songsasen, 2012). Enrichment of IVM media with non-essential and essential amino acids increases mRNA amounts in bovine oocytes and therefore maturation success and developmental potential of the oocyte (Watson et al., 2000).

4.1.5 Lipid metabolism

Environment of the organism impacts its lipid metabolism. A wide variety of factors influencing the fertility are described in the literature: seasonal changes, feeding, reproductive stages (e.g. pregnancy or puerperal period), lactational stage and milk yield (De Rensis and

Scaramuzzi, 2003; Roche, 2006). The bovine species is an interesting model, as dairy cows need to conceive in a period of metabolic stress through lactation.

Effect of environment and nutrition on metabolism and oocytes is well described (Aardema et al., 2011; Alves et al., 2014; Alves et al., 2013; Leroy et al., 2008; Leroy et al., 2004; Zeron et al., 2001).

As reviewed by Leroy et al. (Leroy et al., 2014), presence and concentration of different fatty acids during maturation influences the oocyte on several levels:

- maintenance of meiotic arrest (Homa and Brown, 1992)
- maturation rate (Marei et al., 2009, 2010)
- developmental competence of the oocyte (Lapa et al., 2011; Marei et al., 2009, 2010; Van Hoeck et al., 2013; Van Hoeck et al., 2011).

Intracellular lipids are utilized in different ways during COC maturation:

- Energy source: Intracellular lipids are an alternative and more effective source of energy than carbohydrates; they produce three times more ATP by fatty acid oxidation (Dunning et al., 2014; Ferguson and Leese, 2006). Beta-oxidation activity during IVM influence the oocytes subsequent developmental potential (Ferguson and Leese, 2006)
- Signalling mediator precursors (McKeegan and Sturmey, 2012): Via binding nuclear receptors, fatty acids can regulate the activity of such receptors and furthermore influence gene expression (Bordoni et al., 2006)
- Plasmamembrane and organelle membranes components (Dunning et al., 2014)

Fatty acids can be free (non esterified fatty acids, NEFAs) or can be stored in different forms of lipids, with variable concentrations during IVM (Kim et al., 2001):

- Triglycerides: Triglycerides are the most common form of intracellular lipids in bovine oocytes (Kim et al., 2001). They are stored as lipid droplets in the ooplasm - coupled with proteins (Dunning et al., 2014; Prates et al., 2014). Lipolysis of triacylglycerols by lipases results in liberation of fatty acids, which will then undergo beta-oxidation for ATP production (Cetica et al., 2002; Dunning et al., 2014; Ferguson and Leese, 2006). Lipolysis increases during maturation, which gently reduces the intracellular lipid store in oocytes and cumulus cells (Dunning et al., 2014; Ferguson and Leese, 1999; Kim et al., 2001).

- Cholesterol: A stable content of cholesterol is described in oocytes during maturation (Kim et al., 2001). Not able to synthesize cholesterol, oocytes depend on cumulus cells that provide cholesterol that is transferred through gap junctions from cumulus cells to oocytes (Su et al., 2008). Cholesterol is collected by cumulus cells from the surrounding environment or can be synthetized de novo after paracrine stimulation with OSFs (Su et al., 2008). Cholesterol, jointly with phospholipids, is involved in membrane composition (McEvoy et al., 2000). Cholesterol is also the precursor of steroid hormones and plays a major role in steroidogenesis (Renaville et al., 2010), which happens in mammalian cumulus during maturation (Assidi et al., 2010; Lucidi et al., 2003; Mingoti et al., 2002).
- Phospholipids: Phospholipids are another lipid form that is present in oocytes during maturation (Kim et al., 2001). These lipids have a structural role in membrane formation, with differences in physical properties depending on their fatty acid composition. The more unsaturated fatty acids are, the more stable is the membrane (MacDonald and MacDonald, 1988). Seasonal differences were reported, with more than double unsatured fatty acids in the oocyte membrane composition in winter than summer (Zeron et al., 2001). Heifers are advantaged in their fatty acids composition in follicular fluid with less satured fatty acids than cows (Bender et al., 2010).

The non-esterified fatty acids (NEFA) rise fast during starvation conditions like negative energy balance (NEB) through endogen production (Jorritsma et al., 2003). The most represented NEFAs in bovine serum as well as follicular fluid are oleic > stearic & palmitic > linoleic acid (Leroy et al., 2005). Concentrations vary during lactation (Leroy et al., 2005), between follicular stages (Renaville et al., 2010), with donor age (Bender et al., 2010) and season (Zeron et al., 2001).

Elevated NEFAs concentration during IVM, simulating the *in vivo* situation during NEB, compromises oocytes developmental potential (Leroy et al., 2005). Effects like delayed progression of oocytes to MII, impacted expansion of cumulus and an increased amount of apoptotic cells were reported (Leroy et al., 2005). These effects were not observable when IVM occurred under NEFA concentrations normally associated with positive energy balance (Leroy et al., 2005).

Cumulus plays an active role as barrier for optimisation of oocyte supply with fatty acids (Lolicato et al., 2015; Vireque et al., 2017). Surrounding cumulus regulates the lipid profile of the oocyte by storing fatty acids as triglycerides itself (Aardema et al., 2013; Lolicato et al.,

2015). This effect can be observed as follicular fluid (oleic > palmitic & stearic acids (Leroy et al., 2005)) and oocytes (palmitic > oleic > stearic acids or palmitic > stearic > oleic acids) present a sensibly different fatty acid composition (Kim et al., 2001).

Beside the role of cumulus cells in lipid metabolism, cumulus-oocyte coupling protects the oocyte from reactive oxygen species (ROS) accumulation and lipotoxicity (Lolicato et al., 2015). Lipotoxicity is the response of cells to excess of lipids, especially saturated: triglycerides droplets and free fatty acids are excessively accumulated, impacting organelles and increasing ROS release (Igosheva et al., 2010; Listenberger et al., 2003; Wu et al., 2012b). ROS cause damages in DNA, proteins and lipids (Cruz et al., 2014; Luderer, 2014; Takahashi et al., 2000). Cumulus suffers from saturated fatty acid exposition to protect the oocyte (Leroy et al., 2005). Cumulus presents changes in stress markers and decreased protein expression when saturated fatty acids are added to maturation media in rodents (Wu et al., 2012b). Saturated fatty acids like palmitic or stearic have also a detrimental effect on bovine oocyte maturation (Leroy et al., 2005).
Opposite, the addition of unsaturated acids shows a protective effect (Aardema et al., 2013): oocyte competence increase and the negative effect of saturated acids may be reversed (Aardema et al., 2011). The ratio of satured/unsatured fatty acids is then determining for the outcome of bovine oocyte maturation (Aardema et al., 2011).

4.1.6 Gas impact

Under physiological conditions, the cell metabolism produces free radicals, which also contribute in regulation of cell functions. They are described as players in meiotic resumption and oocyte developmental competence (Blondin et al., 1997).
A particularly active metabolism implies a collateral increase of reactive species production, as it is the case by oocyte and somatic follicular cells during maturation.

Two types of such free radicals are described (Agarwal et al., 2012; Agarwal et al., 2005) as:

- Reactive oxygen species (ROS) like superoxide, hydrogen peroxide or hydroxyl radicals.
- Reactive nitrogen species (RNS) like nitric oxide (NO) or nitrogen dioxide.

These unstable molecules may alter further molecules and induce oxidative stress, dysfunctions or death of cells. This can impair fertility.
An increased ROS production occurs around the ovulation process, where antioxidants are necessary to enable the cells to deal with such an oxidative environment (Agarwal et al., 2005; Devine et al., 2012). Under normal conditions, the production of ROS and the regulation of defence mechanisms are well balanced in the organism in order to avoid damage through oxidative stress.

Antioxidants convert ROS into H_2O (Agarwal et al., 2005). According to Dumesic et al. (2015) (Dumesic et al., 2015) they can be divided in two groups:

- Enzymatic antioxidants like superoxide dismuthase, catalase, glutathione peroxidase, glutathione reductase, different peroxidases and peroxiredoxins.
- Non-enzymatic antioxidants like N-acetylcystein (from the aminoacid L-cysteine), vitamins C and E, elements like selenium or zinc, glutathione and beta-carotene.

Interacting with each other, antioxidants from both groups work against oxidative stress and avoid cellular apoptosis (Ahlemeyer et al., 2001). With regard to reproduction they are therefore able to promote the fertilization process (Dumesic et al., 2015).
Oocytes are very sensitive towards free radicals and the balance in the Redox-System can affect the further oocyte quality, positive or negative. Extrinsic factors are able to challenge the equilibrium in the Redox-System. Factors like environment, nutrition, health status or age of the donor influence the oxidative status of the female gamete (Combelles et al., 2009). Thus, follicular fluid redox status permits to predict the fertilization outcome for the corresponding oocytes (Bedaiwy et al., 2012; Das et al., 2006; Palini et al., 2014). A lack of antioxidants in the female gametes environment imbalances the redox state and impairs oocyte quality and their further developmental competence (Oyawoye et al., 2003). Protective action of antioxidants is described with beneficial effect on oocytes quality and development potential (Cruz et al., 2014; Ikeda et al., 2000; Ikeda and Yamada, 2014). Supplementation permit to increase oocyte quality in subfertile donors like aged ones (Luddi et al., 2016), where the function of the antioxidant system is reduced (Eichenlaub-Ritter et al., 2011; Tarin, 1996).

The oocyte itself stores antioxidants during growth and maturation (Combelles et al., 2009) but cumulus-oocyte coupling also contribute to oocyte protection. The variation of external factors on COC metabolism seems to be balanced at least partially by the cumulus cells. Cumulus cells play a major role in oocyte protection against the deleterious effects of oxidative stress (Tatemoto et al., 2000). Even when maturation rates were reduced in COCs matured together with ROS, more deleterious effects were to observe in denuded oocytes than in cumulus enclosed oocytes under similar conditions (Tatemoto et al., 2000). Cumulus cells stimulate the synthesis of the antioxidant glutathione in bovine oocytes during maturation (de Matos et al., 1997). Also peroxiredoxin-6, involved in antioxidant defence, is upregulated in cumulus cells and in oocytes when these cells are in direct contact with each other (Leyens et al., 2004). Without direct contact, peroxiredoxin-6 is only upregulated in cumulus cells, due to paracrine factors secreted by the oocyte (Leyens et al., 2004).

Still, maturation conditions have a high impact on successful maturation of the oocyte. The major deviation from healthy, physiological maturation condition is the maturation of oocytes *ex vivo*. Even after years of successful maturation and fertilisation *in vitro* the developmental competence of these oocytes is far behind their *in vivo* counterparts. The non-physiologic *in vitro* maturation conditions cause a misbalance via increased ROS production and reduced defence mechanisms (Eichenlaub-Ritter et al., 2011).

4.2 Deficiencies of Cumulus-Oocyte-Complex maturation *in vitro*

Compared to the *in vivo* process, the *in vitro* production of embryo is limited in its efficiency. Even when about 90% of immature bovine oocytes reach metaphase II under *in vitro* conditions (Paula-Lopes et al., 2007), no more than 40% develop to the blastocyst stage (Salhab et al., 2013; van de Leemput et al., 1999). This rate is substantially lower compared to IVP from *in vivo* matured oocytes in the control group, where 73% of blastocysts were recorded (Salhab et al., 2013). Blastocysts expansion rates were also reduced after maturation *in vitro*: only 12% of the total blastocyst count expanded when COCs were matured under *in vitro* conditions compared to 41% expanded blastocysts when they originated from *in vivo* matured bovine COCs (Dieleman et al., 2002). Also for nuclear transfer, where only the recipient cytoplasm originates from IVM matured cells, the development potential is reduced compared to embryos produced via nuclear transfer with *in vivo* matured cytoplasm (Akagi et al., 2008).

From the beginning of IVP, scientists searched for the limiting step. Even when calves could be produced from *in vitro* matured oocytes (Newcomb et al., 1978), the high reduced development potential in blastocyst of bovine oocytes from IVM compared to their *in vivo* matured counterparts was already observed. After oviductal transfer of *in vitro* or *in vivo* matured oocytes before insemination (Trounson et al., 1977) or just after IVF (Greve et al., 1987), the *in vitro* matured group presented a limited blastocyst rate compared to the *in vivo* matured group (Trounson et al., 1977). *In vitro* maturation is well described in the literature as having a detrimental influence on post fertilisation development of oocytes (Dieleman et al., 2002; Humblot et al., 2005; Leibfried-Rutledge et al., 1987; Marquant-Le Guienne et al., 1989; Rizos et al., 2002; Salhab et al., 2013; van de Leemput et al., 1999). A higher rate of developmental abnormalities is described in embryos derived from *in vitro* production (Viuff et al., 1999), this seems to be due to the maturation conditions (Dieleman et al., 2002).

Morphological differences after maturation between *in vivo* and *in vitro* matured COCs are observable with differences in cumulus expansion (Chen et al., 1990; Dieleman et al., 2002; Greve and Callesen, 2001; Salhab et al., 2013) or in mitochondrial distribution in the oocytes (Bavister and Squirrell, 2000; Liu et al., 2010). Beside this, non-directly visible differences like impaired metabolic activity, gene expression pattern in oocytes and surrounding cumulus cells were described (Katz-Jaffe et al., 2009a; Krisher, 2013; Lonergan et al., 2003a; Salhab et al., 2013).

Differences in metabolic pathways occur: for example beta-oxidation is reduced in cumulus cells and oocytes after *in vitro* maturation compared with *in vivo* maturation in several species (Dunning et al., 2014).

The overall gene expression in cumulus differs between the two maturation conditions, as reported in the bovine species (Brisard et al., 2014). Higher RNA expression was found for several genes in MII oocytes and in cumulus cells after IVM than after *in vivo* maturation (Brisard et al., 2014). Expression of genes involved in cell-to-cell interaction, cell cycle, and lipid metabolism was affected after *in vitro* maturation (Brisard et al., 2014). A study interrogating gene expression in mouse cumulus indicates also differences between *in vivo* and *in vitro* maturation, regarding carbohydrate and amino acid metabolism, cell growth, proliferation, function, communication and processes involved in cell death (Kind et al., 2013).

A spontaneous apoptosis process was observed in bovine cumulus cells after IVM (Ikeda et al., 2003). Ikeda and coauthors related this *ex vivo* finding to the available literature in the *in vivo* counterpart, where an absence of apoptosis was reported (Ikeda et al., 2003).

The different microenvironment:

As described by different authors: the *ex vivo* maturation occurs in an abnormal and unnatural way (Gilchrist and Thompson, 2007; Krisher and Bavister, 1998). Differences in the environment of the oocyte impacts *in vitro* maturation success:

- The surrounding cumulus:

  The direct microenvironment of the oocyte has to be conserved to optimize IVM. The importance of cumulus was presented in previous chapters. Preserving a dense surrounding for the oocyte, the presence of cumulus during IVM is linked with better development potential of the oocyte (Auclair et al., 2013; Cetica et al., 1999a). Denuded oocytes present a highly reduced maturation success than intact bovine COCs after IVM (Cetica et al., 1999a).

- The maturation medium:

  The liquid environment of the COC in IVM is highly different as the maturation medium differs from the adapted and dynamic follicular fluid *in vivo* (Orsi et al., 2005). Media for IVM were initially developed for somatic cells culture and underwent empirical adaptations (Gordon, 2003b). TCM-199, already cited in the 1980s, is still nowadays the base of the most widely used medias for bovine oocytes (Gordon, 2003b; Hudson et al., 2014). Many studies focus on the adaptation of maturation media and their impact on the development potential of the oocyte (Albuz et al., 2010; Dovolou et al., 2014; Furnus et al., 1998; Gilchrist and Thompson, 2007; Ha et al., 2015; Paula-Lopes et al., 2007; Phongnimitr et al., 2013; Rose and Bavister, 1992; Rose-Hellekant et al., 1998; Russell et al., 2006; Sugimura et al., 2014; Sutton et al., 2003b; Ulloa et al., 2014).

  In the static *in vitro* system, metabolites for the whole maturation process are provided from the beginning of maturation (Sutton-McDowall et al., 2010). The maturation may be disturbed as energy substrates in the medium impact meiotic resumption, by activation or suppression, depending of their presence (Downs, 2015; Sutton-McDowall et al., 2010; Thompson, 2006; Thompson et al., 2007). An impact of the

composition of metabolites in the surrounding COC environment on the communication between cumulus and oocyte was reported (Hudson et al., 2014).

The increased production of ROS:

The *in vitro* conditions also challenge the oxidative equilibrium via increased production of free radicals and reduced defence. *In vitro* conditions favour an increased ROS production (Cetica et al., 2001) due to:

- Increased oxygen concentration:

  In the female reproductive tract, the COCs deal with an environment massively reduced in oxygen (Mastroianni and Jones, 1965; Van Blerkom, 1998). Under *in vitro* conditions, using 5% CO_2 in air, oxygen concentration is about twenty times higher (Agarwal et al., 2006; Eichenlaub-Ritter et al., 2011). Hyperoxic conditions was described as factor increasing ROS production in bovine embryos and altering defensive response to oxidative stress (Guerin et al., 2001). Also in bovine species, a lower oxygen concentration during IVM would improve further competence of the COC (Bermejo-Alvarez et al., 2010; Hashimoto et al., 2000a).
- Exposition to visible light:

  In opposite to the complete darkness in *in vivo* maturation conditions, *in vitro* matured COCs are in alternation with dark incubator conditions punctually exposed to light during handling. This short light expositions are known to induce production of ROS and DNA damage in other cell types (Beehler et al., 1992).
- Metallic cations:

  Cations like Fe or Cu ions that are present in the media may induce production of ROS (Guerin et al., 2001).
- Atmospheric pollutants:

  Filtration permit to avoid concentration of such pollutants in the incubator atmosphere (Guerin et al., 2001).
- Overall media composition:

  As previously reported, high concentrations of metabolites from the beginning of IVM may negatively impact the COC outcome (Combelles et al., 2009). Environment may influence metabolism toward pathways that produce particularly high ROS amount. High glucose concentrations typically induce increased ROS production via glycolysis and oxidative phosphorylation for ATP production (Combelles et al., 2009).

The reduced antioxidative system:

In vitro conditions present also reduced antioxidative defence for oocyte compared to *in vivo* conditions (Guerin et al., 2001):

- *In vitro* matured and *in vivo* matured oocytes present a different expression of antioxidant genes (Lonergan et al., 2003a). In the porcine species, a highly reduced antioxidant concentration in oocytes after *in vitro* maturation compared to *in vivo* maturation was reported (Brad et al., 2003).
- *In vivo*, antioxidant supplementation to the COC is ensured via cumulus cells and follicular fluid (Tatemoto et al., 2004; Tatemoto et al., 2000).
  COCs that matured *in vivo* in aged donors present a particular situation due to reduced antioxidant expression in cumulus (Matos et al., 2009). These aged oocytes own then a reduced defence mechanism against increased free radicals, gas variations and suboptimal culture conditions (Eichenlaub-Ritter et al., 2011).

The oxidative stress due to increased ROS and/or reduced antioxidant production can induce arrest of maturation, altered spindle morphology, DNA damage, aneuploidy, apoptotic signals and reduced oocytes developmental competence (Bierkamp et al., 2010; Eichenlaub-Ritter et al., 2011; Hu et al., 2001; Tatemoto et al., 2000). Combelles and coworkers suggested also an influence of oxidative stress on gene expression during oocyte maturation (Combelles et al., 2009). Reduction of oxygen concentration or addition of antioxidants is able to limit or reverse already described damages (Choi et al., 2007; Hu et al., 2001).

Beneath the described influence of the microenvironment, the macroenvironment plays also a critical role. Lab environment can house disturbers like cytotoxic materials, variations in water quality, medias, gas and other physical or chemical factors. They can impact the cells in culture and are nicely reviewed in the literature (Boone and Shapiro, 1990; Higdon et al., 2008; Schiewe et al., 1990).

Research is ongoing for a better comprehension of these suboptimal results and optimisation of this rate limiting first step of IVP of embryos. Another hypothesis for the reduced developmental competence of *in vitro* matured oocytes is related to the mRNAs stored in bovine oocytes, transcribed already prior maturation (Wrenzycki et al., 2007). Oocytes with different developmental competence present already a different poly(A) tail prior to

maturation (Brevini-Gandolfi et al., 1999). Inadequate maturation conditions compared to adequate ones may also decrease competence of oocytes evaluated as good prior IVM via impacting mRNA polyadenylation (Gandolfi and Gandolfi, 2001) and therefore affects gene expression regulation (Lonergan et al., 2003b). Impact of *in vivo* and *in vitro* maturation conditions on mRNA content in oocytes was already compared (Jones et al., 2008). Jones and coauthors observed the overexpression of numerous genes in oocytes after IVM compared to *in vivo* maturation (Jones et al., 2008). They suggest a dysregulation in gene transcription or in post-transcriptional modifications under *in vitro* maturation conditions to explain this overexpression and the parallel lower competence of such oocytes (Jones et al., 2008).
Selection of the COCs, which still develop successfully after IVM, would help to improve the IVP process and optimize the clinical use.

4.3 Cumulus cells as non-invasive biomarkers for oocyte developmental competence

For *in vitro* production of embryos, reliable tools are required to select oocytes for their developmental competence (here defined as the ability to develop until the blastocyst stage *in vitro*). The traditional selection criteria for COCs are based solely on morphological grading using light microscopy.
Preselecting competent oocytes before maturation is described based on:

- cumulus morphology (Blondin and Sirard, 1995; Hawk and Wall, 1994; Laurincik et al., 1996; Shioya et al., 1988)
- oocyte/cytoplasm appearance (Hawk and Wall, 1994; Nagano et al., 1999)
- oocyte staining with brilliant cresyl-blue (Silva et al., 2013).

After maturation, selection of COCs according maturation success was, up to now, based on morphologic criteria like:

- cumulus expansion (Hunter and Moor, 1987)
- mitochondrial distribution (Bavister and Squirrell, 2000; Liu et al., 2010)
- observation of meiotic spindle (Caamano et al., 2013; Wu et al., 1997)
- zona pellucida birefringence (Held et al., 2012)
- presence of a polar body (Park et al., 2005).

Except of cumulus expansion, all these criteria are investigated on denuded oocytes. Denudation is part of the ICSI (intracytoplasmic sperm injection) procedure. This is in the

human and equine species the gold standard for fertilization of oocytes *in vitro*. For cattle, classical IVF is the most effective way for IVF (Galli et al., 2014), which requires cumulus-enclosed oocytes. Therefore, the solely morphological grading of COCs is mainly based on cumulus appearance and expansion, which is not sufficient to ensure correct selection of normal matured bovine oocytes (Vassena et al., 2003). Therefore, additional markers are needed for a better selection of intact COCs for development potential (Wang and Sun, 2007). Researchers focussed in different species on culture media, follicular fluid or surrounding somatic cells as non-invasive biomarker sources for developmental competence of the corresponding oocyte (Anderson et al., 2009; Assou et al., 2008; Chen et al., 2016; Cillo et al., 2007; Feuerstein et al., 2007; Hamel et al., 2008; Hamel et al., 2010; McKenzie et al., 2004; Robert et al., 2001; Seli et al., 2010; van Montfoort et al., 2008; Zhang et al., 2005). These bio sources were searched for markers on gene expression and metabolic level and correlated to the oocytes developmental competence. Granulosa cells recovered from transvaginal aspiration of follicular fluid during bovine ovum pick up were described as non-invasive marker source for oocyte competence, with particular gene expression for the ones surrounding competent oocytes (Robert et al., 2001).

Some studies are already available that focus on cumulus cells as non-invasive biomarkers. A specific oocyte selection based on cumulus cell biopsy will present the non-negligible advantage of non-invasivity parallel to high predictive accuracy due to the tight contact and exchanges within the COC (Li et al., 2008; Pourret et al., 2016).
Cumulus cells examination presents also a rich information source about the COC with a higher protein expression compared to the oocyte (Memili et al., 2007; Peddinti et al., 2010). Non-invasivity was already proven in a study, where biopsies of single bovine cumulus detected gene biomarkers for developmental competence of the corresponding oocyte (Bunel et al., 2015). The oocyte remains intact while cumulus is consumed for examination; this allows a further clinical use of these oocytes selected after selection via cumulus biomarkers.

Fewer studies were published about transcriptomics compared to proteomics, regarding the COC. The analysis of the proteome does not include enrichment steps, as it is possible for transcriptomic studies. The proteomics analysis of minute cumulus amounts – even in pooled samples - is therefore facing special technical challenges.
The transcriptomic studies observed different gene expression profiles around maturation that correlated with different biological groups:

- Maturation Stages:

  Gene expression differed in cumulus at different stages of maturation in bovine (Bunel et al., 2013) similar as in other mammals (Anderson et al., 2009; Assou et al., 2006; Lee et al., 2011; Ouandaogo et al., 2011).

- Maturation Condition:

  Gene expression in cumulus cells is altered by different maturation conditions:

  - Differences were observed between *in vivo* and *in vitro* maturation in bovine (Assidi et al., 2010; Assidi et al., 2008; Assidi et al., 2013; Burmester-Kintrup, 2014; Salhab et al., 2013; Tesfaye et al., 2009) and primate (Lee et al., 2011; Ouandaogo et al., 2012).
  - Variation in *in vitro* maturation condition that resulted in altered cumulus gene expression:

    - intact COCs versus oocytectomised complexes (Regassa et al., 2011)
    - heat stressed COCs versus non-heat stressed COCs (Rispoli et al., 2013)
    - different gas concentrations (Bermejo-Alvarez et al., 2010) or different oil overlay on the medium (Burmester-Kintrup, 2014).
  - The expression of certain genes remained similar in cumulus after different maturation conditions (Assidi et al., 2010; Assidi et al., 2013).

- Developmental Competence:

  Gene expression in bovine cumulus differs, when housing a competent or a non-competent oocyte (Assidi et al., 2010; Assidi et al., 2008; Bettegowda et al., 2008; Bunel et al., 2015; Bunel et al., 2013; Matoba et al., 2014; O'Shea et al., 2012). Similar results were obtained in other mammals (Anderson et al., 2009; Assidi et al., 2011; Ekart et al., 2013; Feuerstein et al., 2007; Gebhardt et al., 2011; Ouandaogo et al., 2011; VandeVoort et al., 2015; Wathlet et al., 2013; Zhang et al., 2005).

  The different cumulus gene expression can discriminate oocytes with different developmental potential, even with similar cumulus morphology (Adriaenssens et al., 2011; Assou et al., 2008; Cillo et al., 2007).

  Correlation of gene expression in human cumulus was reported to:

  - Fertilisation success (Anderson et al., 2009; Bergandi et al., 2014; van Montfoort et al., 2008)
  - Embryo quality (Anderson et al., 2009; Assou et al., 2008; Cillo et al., 2007; McKenzie et al., 2004; Wathlet et al., 2011)

- Pregnancy prediction (Assou et al., 2008; Iager et al., 2013; Wathlet et al., 2013)
- Outcome of pregnancy (Assidi et al., 2011)
- Chromosomal aberrations in the oocyte (Fragouli et al., 2012).

In summary, several studies on gene expression level detected alterations in the cumulus for different maturation stage, conditions and developmental competence. Studies on cumulus level, especially for single cumulus complexes, are up to now limited to the analysis of global protein expression in cumulus (Dieleman et al., 2002; Hamamah et al., 2006). Nevertheless, investigation of the cumulus proteome reveals changes that are more close to the cumulus phenotype compared to the analysis of cumulus gene expression (Anderson and Anderson, 1998). The advantages of a proteomics approach will be reviewed in the next chapter.

4.4 The COC proteome

4.4.1 Advantages of proteomic studies

As presented in the previous chapter, an increasing amount of Omics studies on COCs is available. Most of them focus on a global analysis of mRNA expression. These transcriptomic studies examine gene expression, but post-transcriptional regulation of RNA, translation process into protein, as well as post-translational modifications of proteins are missed (Figure 2). Transcriptomics don't inform precisely and completely about functions and biological processes coded by the genome expression, as protein would do. An example for this situation is the silent transcriptional activity in oocyte during maturation (De La Fuente et al., 2004). Presence of mRNA is due to previous transcription during oocyte growth and storage. Still, oocytes possess translational activity during maturation (Chen et al., 2011).

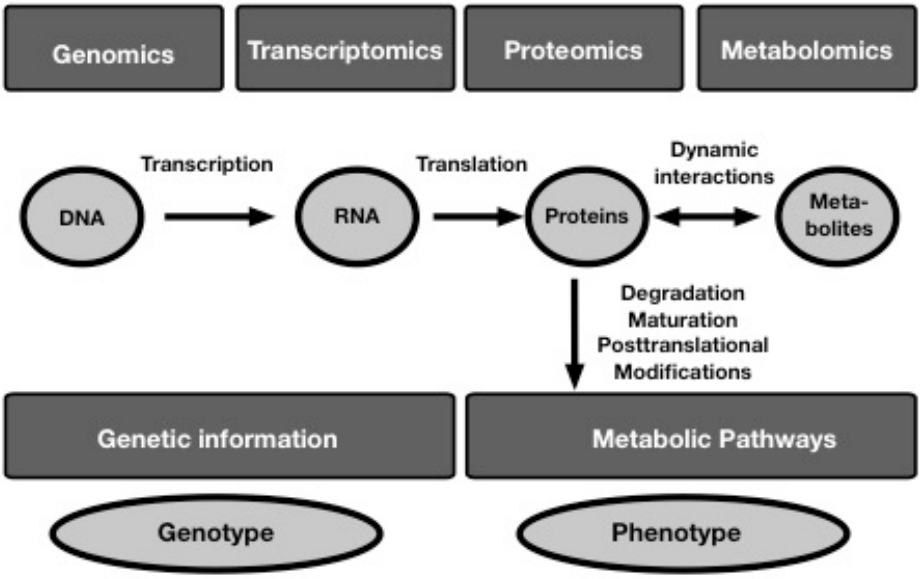

Figure 2: Scheme for the different –omics-levels: Proteomics offers information that is correlated more closely to the cells phenotype compared to transcriptomics. Proteomics can also consider post-transcriptional regulation of RNA expression, the translation process into proteins as well as post-translational modifications of proteins. All these facts are missed in transciptomics-only studies (modified from https://grantfundingresources.wordpress.com/tag/omics-phenotypes/).

Protein identification, quantitative and qualitative protein expression, localisation of this expression, protein activity and protein degradation cannot be predicted from transcriptomic data completely (Arnold and Frohlich, 2011; Bhojwani et al., 2006; Wolf et al., 2006).
During maturation, the reduced transcriptional and increased translational activity (Tomek et al., 2002) suggest that proteins play a central role in the maturation process of the COC (Bhojwani et al., 2006). A detailed characterization of the COC proteome would contribute to a better comprehension of the maturation step.

Due to post-transcriptional and post-translational modifications, over one million proteins can be expected from about 22000 genes that are contained in the bovine genome (Burt, 2009; Katz-Jaffe et al., 2009b). Different cells are able to express a unique proteome of the very same genome, adapted to their needs and actual conditions, which is influenced by internal and external stimulations.
The protein expression in a specific sample material, for particular condition, is called proteome. Studies on protein level are an important goal in understanding oocyte maturation,

as by the use of proteomics the end product of the interaction of genes and proteins with environmental factors are investigated.

4.4.2 Proteomic studies in COCs

Different techniques were used to examine protein content in the COC.

Proteomic analysis of oocytes

In older studies electrophoresis (Coenen et al., 2004; Kastrop et al., 1990a, b, 1991) and in the last ten years mass spectrometry (Berendt et al., 2009; Bhojwani et al., 2006; Demant, 2012; Massicotte et al., 2006) were used in several studies to examine the oocyte. All of these cited studies used pooled bovine oocytes samples. In 2016, the first study analysing proteins in single human oocytes (Virant-Klun et al., 2016) and recently in single bovine oocytes (Labas et al., 2018) were published.

Proteomic analysis of cumulus

For the investigation of cumulus proteins, a similar evolution of techniques can be observed. Many studies used electrophoresis with radioimmuno assays (Bovine: (Dieleman et al., 2002; Wu et al., 1996); Human: (Hamamah et al., 2006)) or western blot (Bovine: (Aparicio et al., 2011; Burmester et al., 2012; Burmester-Kintrup, 2014; Mohan et al., 2003; Salhab et al., 2013) Human: (Bergandi et al., 2014)). In recent years, papers using mass spectrometry based proteome analysis for bovine cumulus (Memili et al., 2007; Peddinti et al., 2010) as well as other mammals (McReynolds et al., 2011; McReynolds et al., 2012; Walter et al., 2014) were published. All of these MS based studies used pooled cumulus samples. Only a very recent publication reports protein examination of cumulus at single oocyte level (Labas et al., 2018).

Several interesting biological observations with regard to cumulus protein expression were observed:

- COC origin impacts the protein expression in cumulus: in human, age of the donor is related to different protein expression in cumulus (McReynolds et al., 2011; McReynolds et al., 2012)
- Cattle oocytes undergoing *in vitro* maturation present variations in protein expression prior, during and post *in vitro* maturation (Berendt et al., 2009; Bhojwani et al., 2006;

Coenen et al., 2004; Kastrop et al., 1990a, b, 1991; Labas et al., 2018; Massicotte et al., 2006).

- The bovine cumulus cell protein synthesis during *in vitro* maturation differs quantitatively and qualitatively from the oocytes (Labas et al., 2018; Wu et al., 1996). Already in immature COC, bovine oocytes and cumulus cells present differences in protein expression (Memili et al., 2007; Peddinti et al., 2010).
- Through different stages of maturation, the total protein content of cumulus increases, and the type of expressed proteins varies (Wu et al., 1996). Some of these proteins are suspected to play a role in the maturation process (Wu et al., 1996).
- Maturation conditions have also an impact on cumulus protein expression: As already described in transcriptomic studies, the expression of proteins in cumulus cells presents variations between the different *in vivo* (Dieleman et al., 2002; Salhab et al., 2013) and *in vitro* (Salhab et al., 2013) maturation conditions (Aparicio et al., 2011; Burmester et al., 2012; Burmester-Kintrup, 2014).
- Protein expression in cumulus differs between different COC morphologies (Walter et al., 2014) and was related to fertilisation outcome of the oocyte (Bergandi et al., 2014).

Due to the amount of material needed for protein analysis – which does not include any enrichment steps - most of the proteomics studies were conducted using pooled samples (Bergandi et al., 2014). Even when pooling occurs just before examination, e.g. based on oocyte outcome, the results remain imprecise. It isn't possible to ensure that all single cumulus samples from a pooled sample present the supposed marker. The difficulties of sample pooling were nicely reviewed in the literature (Diz et al., 2009; Telaar et al., 2010). More and more techniques develop to examine minute sample amounts (Feist and Hummon, 2015).

Nowadays, technical evolution provides more sensitive techniques for low abundant proteins and smaller amount of material like cumulus biopsies. This allows the analysis of samples corresponding to single oocytes, which provides the unique opportunity to correlate the cumulus proteome to single oocytes with all the related facts like origin, age, follicular morphology or developmental competence. For classical *in vitro* fertilisation the cumulus is at

least partially necessary. The analysis of cumulus parts, without complete denudation, gives the opportunity to further fertilisation of the oocyte after sampling.

5 Material and Methods

5.1 Preparation of oocyte donor heifers

Six pure Brown Swiss heifers between 1 year and 9 months and 2 years and 8 months were used for this study. The heifers came from the same alpine region, were fed with grass silage, grass and hay after standard weaning time, all of them had a similar Body Condition Score (3-3.5). All heifers were oestrous synchronised before entering the final project phase.

Synchronisation procedure:

All Brown Swiss heifers (n=6) used in this study were processed in October and November 2014. They were checked for healthiness and cyclic activity before the start of treatment. Cycle synchronization was achieved by two injections of Luprostiolum 11 days apart (15mg/animal, intramuscularly; Prosolvin, Virbac, Glattbrugg, Switzerland). Oestrous was expected 2-3 days after the last PGF2 injection (Noakes, 1986). Follicular development and ovulation were supported using an intramuscular injection Gonadorelinum (0.25mg/animal, intramuscularly, Fertagyl, MSD Animal Health, Lucerne, Switzerland) 48 hours after the last prostaglandin injection. Successful ovulation expected after 24 hours was controlled by the disappearance of the dominant follicle and later by presence of corpora lutea by ultrasonography.

Superovulation procedure:

For the *in vivo* maturation of COCs, half of the heifers (n=3) underwent a superovulation treatment with ovulation induction according to the protocol described here. The other three served as donors of immature COCs for *in vitro* maturation.

At day 9.5 of the new cycle, the dominant follicle (>1cm) was aspirated transvaginally for synchronisation of the follicular wave, as the response to superovulation is described to be reduced in presence of large palpable follicles (Lima et al., 2007). Dominant follicle aspiration was chosen as alternative to estradiol for follicular wave synchronisation (Mapletoft and Bo, 2011) because estradiol compounds are not registered in the European Union. For optimisation of the response to equine Chorion Gonadotrophin (eCG) and to synchronize ovulation, the heifers received an intravaginal progesterone-releasing device (Vos et al., 1994).

Superovulation was initiated in three heifers at day 9.5 using equine Chorionic Gonadotrophin (eCG, 2500 Units/animal, intramuscularly, Folligon, MSD Animal Health, Lucerne) (Mapletoft and Bo, 2011; Mapletoft et al., 2002). At the same time, a mid-luteal progesterone level was ensured using a progesterone releasing intravaginal device (Prid® delta, Biokema, Crissier, Switzerland) in all heifers, to take advantage of the rebound effect after removal for oestrous induction (Vos et al., 1994). Corpora lutea regression was induced in all the six heifers with 15mg Luprostiolum injections (15mg/animal, intramuscularly; Prosolvin Virbac , Glattbrugg) at 48 and 60 hours after Prid® insertion.

In the three superovulated heifers, the Prid® was removed at day 5 after eCG injection. The response to superovulation was evaluated by ultrasonography. At the same time the cows received an intramuscular injection of 0.25mg Gonadorelinum (Fertagyl, 0.25mg/cow intramuscular, MSD Animal Health, Lucerne,) to induce an LH surge. The peak of the preovulatory LH surge was expected three hours after injection (Bordignon et al., 1997), which was therefore defined as start for the *in vivo* maturation. The heifers were slaughtered 24 hours after the last Gonadorelinum injection.

The other three heifers served as donors for the *in vitro* maturation group. These animals were synchronised but not superovulated. Slaughtering was scheduled 6 days after the PRID insertion, without removal of the device.

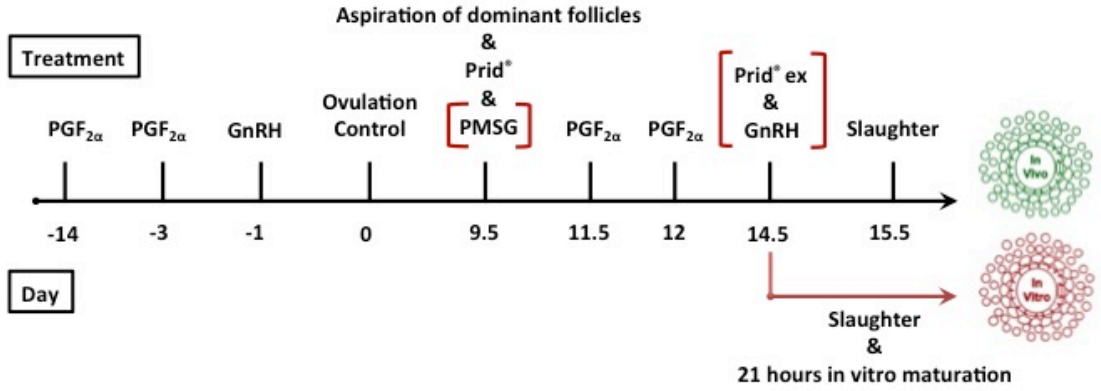

Figure 3: Simplified scheme of synchronisation and superovulation treatments for the collection of COCs after *in vivo* and *in vitro* maturation

5.2 Collection of COCs from the slaughtered heifers

Figure 3 gives a brief schematic overview of all the performed treatments to obtain *in vivo* and *in vitro* matured oocytes. After slaughtering, ovaries were extracted from their carcasses

<5 minutes after slaughtering. All ovaries were hold <30 minutes after excision in NaCl 0.9% at 35°C containing antibiotics (Annex 1-1). Each ovary was sliced separately into a glass dish containing phosphate-buffered saline solution (PBS; Annex 1-2). In non-superovulated heifers dominant follicles were discarded to obtain COCs of similar stage. The medium was searched for COCs under the inverted microscope.

In vivo maturation group:

Assessment of cumulus quality was performed as described in the literature (Dovolou et al., 2014) by classification of the COCs based on the degree of cumulus expansion. Successfully matured COCs completed expansion of the surrounding cumulus cells (Figure 4, right picture), in contrast to immature (Figure 4, left picture) or "failed to mature" COCs (Figure 5) with tight cumulus layers adhering to the zona pellucida. Moreover completed maturation was assessed on the denuded oocytes. Extrusion of the first polar body was controlled under the stereomicroscope. Collected COCs were stored in TCMair (Annex 1-3) at 38.5°C up to further processing.

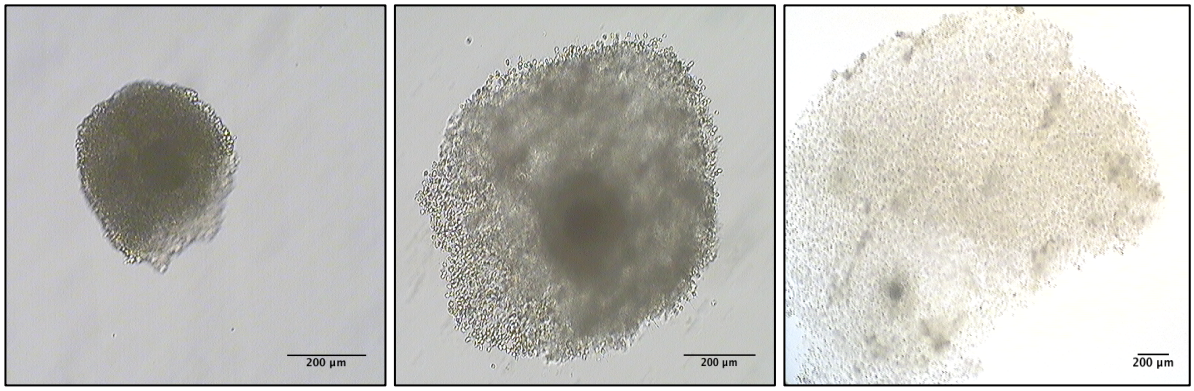

Figure 4: Left: Immature COC with several layers of compact cumulus in comparison with the same COC after successful *in vitro* maturation (middle). Middle: *in vitro* matured COC with several layers of expanded cumulus matured in an individual maturation drop. Right: *in vivo* successfully matured COC, with numerous layers of expanded cumulus, as recovered from heifers slaughtered after superovulation.

In vitro maturation group:

For the collection of successfully *in vitro* matured COCs and not successfully matured COCs after 21 hours of IVM, COCs were collected from the synchronised non-superovulated heifers.

COCs were separately washed in preIVM washing drops (TCM-BSA; Annex 1-4) and each COC was placed in an individual maturation drop (TCM-BSA+hormones; Annex 1-5). After an equilibration period of 3 hours pictures were taken of each COC in his maturation drop (pre IVM picture). The dishes were incubated 21 hours at 38.5 °C and 5% CO_2. Quality of cumulus expansion was evaluated and graded according to the *in vivo* matured COCs 21 hours after initiation of maturation (Figure 4).

5.3 Cumulus collection and preparation for proteomic analysis

Samples from synchronised/superovulated heifers that successfully matured or failed to mature in vivo:

Each ovary of the superovulated animals was sliced in a separate TCM Air (Annex 13.1.3) dish (petri dish 60x15 mm (item n°628160), Greiner bio-one GmbH, Frickenhausen, Germany). An overview on the collected COCs for each heifer is given in Table 1. Before further washing in 3 subsequent TCMair drops (100 µl each drop) a picture of each complete COC was taken. Each COC was stored and washed in individual drops of TCMair. The transfer of COCs was conducted using a Stripper micropipettor (The Stripper (item n° MXL3-135), Origio a/s, Måløv, Denmark) adjusted to 2.5 µl volume. In the last TCMair drop the oocyte was denuded from their cumulus and an equal volume of 2.5 µl cumulus cells was transferred in a sample-drop of 100 µl PBS-PVA (Annex 1-6), a new picture of the cumulus sample in PBS-PVA was saved (Figure 5, right). The cumulus was washed in three further 100 µl drops of PBS-PVA, transferred also in a constant volume of 2.5 µl using the Stripper pipette. From the last PBS-PVA washing drop the cumulus samples were aspirated in a volume of 2.5 µl and placed in labelled tubes (SafeSeal tube 1.5ml, Ref 72.706, Sarstedt, Nümbrecht, Germany). The sample tubes were snap frozen in liquid nitrogen and stored until further proteomics analysis. The corresponding oocyte, kept in the last TCMair drop, was fully denuded in trypsin solution (Trypsin 1:5; Annex 1-7) for further evaluation of final maturation. Therefore, the oocytes were controlled under the inverted microscope for extrusion of the first polar body. Negative controls for TCMair were prepared as described above without containing cumulus cells: medium free of cumulus cells was washed 3 times in TCMair as well as three times in PBS-PVA (using a Stripper pipette adjusted to 2.5 µl). From the last PBS-PVA wash drop a volume 2.5 µl was collected as media control sample and also snap frozen in liquid nitrogen. All tubes were stored at -80°C up to processing the samples for proteomics analysis.

Table 1: Superovulation response of the heifers in the "*in vivo*" maturation group and selection of the analysed samples

	Heifer 1	Heifer 2	Heifer 3
Superovulation response	Good	Poor	Good
Number of corpora lutea	1	1	1
Number of dominant follicles	>20	5	>15
Number of successfully matured COCs	9	2	20
Number of successfully matured COCs stored for analysis	9	2	13
Number of COCs that failed to mature	35	2	7
Number of COCs that failed to mature stored for analysis	15	2	5
Number of successfully matured COCs used in the final analysis	4	1	0
Number of COCs that failed to mature used in the final analysis	4	0	1

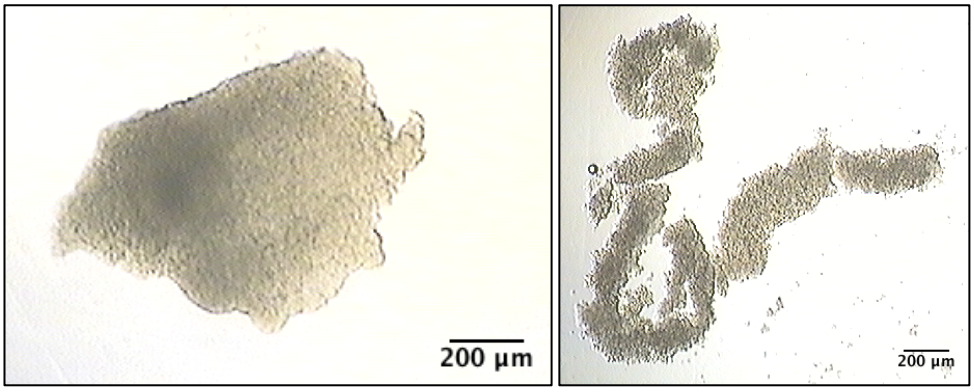

Figure 5: Left: COCs that "failed to mature" *in vivo*, with tight cumulus, from a synchronised/superovulated heifer. No polar body was observed in the corresponding oocytes. Right: corresponding tight " failed to mature" cumulus, collected for the proteomics analysis.

Samples from synchronised non-superovulated heifers of the "in vitro" group that matured successfully or failed to mature after 21 hours of IVM:

Each ovary of the not superovulated animals was sliced in a separate TCM Air (Annex 13.1.3) dish. COCs were washed individually in preincubated four 100 µl TCM-BSA drops (Annex 1-4) and transferred in individual 30 µl maturation drops. An overview on the collected COCs for each heifer is given in Table 2. After 21 hours *in vitro* maturation in individual drops, a

picture was taken from each COC and the individual COCs were placed from the IVM drop in individual TCMair drops. Each COC was washed in 3 subsequent 100 µl TCMair drops, using a Stripper pipette adjusted to 2.5 µl volume. In the last TCMair drop the oocyte was denunded using a 2.5 µl Stripper pipette. A volume of 2.5 µl cumulus cells in TCMair was transferred in individually labelled 100 µl PBS-PVA drops (Annex 1-6). A picture of the cumulus sample in these drops was taken and the cumulus washed in 3 subsequent 100 µl PBS-PVA drops (transfer in 2.5 µl Stripper pipette). The cumulus was aspirated from the final PBS-PVA wash drop in a volume of 2.5 µl and transferred in a 1.5 ml labelled tube (SafeSeal tube 1.5ml, Ref 72.706, Sarstedt, Nümbrecht, Germany). The samples were snap frozen and stored in liquid nitrogen until further analysis. Corresponding oocytes were fully denuded in trypsin, and checked for presence of a polar body under the inverted microscope. Media controls were prepared accordingly as described for the *in vivo* matured COCs. The tubes were stored at -80°C up to processing the samples for proteomics analysis.

Table 2: COC harvest in the heifers of the "*in vitro*" maturation group and selection of the analysed samples

	Heifer 4	Heifer 5	Heifer 6
Number of corpora lutea	1	1	1
Number of dominant follicles	1	1	2
Number of collected COCs of good/middle/bad quality	9/10/16	17/1/17	6/5/27
Number of successfully matured COCs of good quality stored for analysis	6	5	4
Number of COCs of good quality that failed to mature stored for analysis	3	8	2
Number of COCs matured successfully *in vitro* used in the final analysis	3	1	1
Number of COCs that failed to mature *in vitro* used in the final analysis	1	3	1

5.4 Proteomic examination

Samples from 20 single cumulus complexes were examined in one single proteomics analysis. An equal repartition was reached by selection of the biggest five cumulus samples for each of the maturation status and maturation conditions with 5 samples in each group (Table 3). The colour code of Table 3 will be used in the results section.

Table 3: Description of the cumulus samples examined in proteomic analysis with the maturation conditions, maturation outcome, COC origin and number in the MS run. The colour code for the 4 groups will also be used in the result section.

Maturation condition	Maturation outcome	Cow	Oocyte	Number of run
In vivo	Successfully matured	1	03	08
		1	04	18
		1	15	21
		1	16	13
		2	3	09
	Failed to mature	1	05	11
		1	07	19
		1	08	07
		1	21	32
		3	11	22
In vitro	Successfully matured	4	01	24
		4	02	14
		4	22	12
		5	18	15
		6	03	28
	Failed to mature	4	04	31
		5	01	27
		5	04	20
		5	05	26
		6	07	25

5.5 Proteomics Analysis

5.5.1 Sample preparation

A combination of sonoreactor based cell lysis (SR, adapted from (Lopez-Ferrer et al., 2005)) and filter-aided sample preparation (FASP; adapted from (Wisniewski et al., 2009)) were used for protein extraction and digestion (SR-FASP). The SR-FASP protocol was established

especially for the preparation of the minute sample amounts of single cumulus complexes (COCs) at the Functional Genomics Center Zurich (Figure 6). As first step samples were treated with four freeze/thaw cycles in 90% methanol. After 15 minutes the collected pellet was solved in 30 µl SDS lysis buffer (4% SDS, 100 mM Tris/HCL pH 8.2, 0.1 M DTT - dithiothreitol) and incubated at 95 °C for 5 min. Afterwards samples were treated with High Intensity Focused Ultrasound (HIFU) for 10 min with an ultrasonic amplitude of 65% in cycle 0.5 (Sonoreactor UTR200; Hielscher Ultrasonics, Teltow, Germany). Samples were centrifuged for 10 minutes at 16000*g* and protein concentration was estimated with the Qubit® Protein Assay Kit (Life Technologies, Carlsbad, Ca, USA). For each sample, 10 µg of proteins were taken and used for on-filter digestion using an adaption of the filter-aided sample preparation (FASP) protocol (Wisniewski et al., 2009). Briefly, proteins were diluted in 200 µl of UT buffer (Urea 8 M in 100 mM Tris/HCL pH 8.2), loaded on a Microcon-30kDa Centrifugal Filter Unit with Ultracel-30 membrane (Merck Millipore, Darmstadt, Germany) and centrifuged at 14,000*g* for 25 minutes at room temperature. The filter unit was washed using 200 µl UT buffer and another centrifugation at 14,000*g* for 25 minutes. For alkylation of reduced proteins 100 µl iodoacetamide 0.05 M in UT buffer were added to the filter unit and incubated for 5 minutes. Three washing steps with 100 µl UT and two washing steps with 100 µl NaCl 0.5 M were performed. Proteins were digested over night on the filter-unit in a wet chamber at room temperature using 120 µl of 0.05 M triethylammonium bicarbonate buffer (pH 8.5) containing trypsin (Promega, Madison, WI, USA) in a ratio 1:50 (w/w). After elution, the peptide solution was acidified using trifluoroacetic acid (TFA) to a final concentration of 0.5%. Peptides were desalted using Finisterre solid phase extraction C18 columns (Teknokroma, Barcelona, Spain), dried and resolubilized in LC-MS solution (3% acetonitrile, 0.1% formic acid) for MS analysis.

5.5.2 Mass spectrometry

Samples were analysed in random order in one analytical run using reverse-phase LC-MS/MS on an Orbitrap Fusion mass spectrometer (Thermo Scientific, USA) data dependent acquisition (DDA) mode (Figure 6). The instrument was coupled to a nano HPLC system (EASY-nLC 1000, Thermo Scientific, Germany).

500 ng of peptides were loaded on a self-made frit-column (75 µm × 150 mm) packed with reverse phase material (ReproSil-Pur 120 C18-AQ, 1.9 μm beads (Dr. Maisch HPLC,

Ammerbuch, Germany), coupled to a fused-silica emitter (20 µm × 8 cm, tip: 10 ± 1 µm; New Objective, Woburn, MA, USA).

Solvent composition was 0.1% formic acid in water for channel A, and 0.1% formic acid in acetonitrile for channel B. Peptides were eluted at a flow rate of 300 nl/min by a gradient of 1 to 25% ACN in 50 min, 25-32% ACN in 10 min and 32-97% in 10 min. Full-scan mass spectra (300–1500 *m/z*) were acquired at a resolution of 120'000 at 200 *m/z* after accumulation to a target value of 4e5. We used look mass correction (371,1010 and 445.12003 m/z) and the maximum cycle time between precursor mass scans was set to 3 seconds. Data dependent MS/MS were recorded in the linear ion trap using quadrupole isolation with a window of 1.6 Da and HCD fragmentation with 30% fragmentation energy. The ion trap was operated in rapid scan mode with a target value of 1e2 and a maximum injection time of 35 ms. Precursor signals were selected for fragmentation with a charge state from +2 to +7 and a signal intensity of at least 5e3. A dynamic exclusion list was used for 25 seconds and maximum parallelizing ion injections was activated. A pool containing 0.5 µl of each sample was analyzed and used as reference for aligning in data analysis.

5.6 Data analysis

Progenesis QI for Proteomics Software (Nonlinear Dynamics, Newcastle upon Tyne, UK) was used for label-free quantification (Figure 7). Automatic aligning was performed against the reference raw-file of the sample pool. Peak picking was performed with enabled high sensitivity option and only peptide ions with the charges 2, 3 and 4 were used for the analysis. The top five tandem mass spectra were exported using charge deconvolution and deisotoping option with a maximum fragment ion count of 200 peaks per MS/MS. The spectra were searched against the Uniprot database for Bos taurus (NCBI taxonomy ID 9913, release date 20140521) that has been concatenated with its reversed sequence information using Mascot Server v.2.4.3 (Matrix Science, London, UK) with a tolerance of 10 ppm for precursor ion mass and 0.5 Dalton for fragment ion tolerance. Enzymatic specificity was set to trypsin allowing a maximum of 2 missed cleavage sites. Carbamidomethylation of cysteine was specified as a fixed modification, and oxidation of methionine, deamidation from glutamine and asparagine and protein n-terminus acetylation were selected as variable modifications.

The mascot search result was loaded into Scaffold v4.1.1 (Proteome Software Inc., USA) to assign protein probabilities by the Protein Prophet algorithm (Nesvizhskii et al., 2003). Proteins that contained similar peptides and could not be differentiated based on MS/MS

analysis alone were grouped to satisfy the principles of parsimony. A spectrum report was exported and loaded into Progenesis QI for proteomics to link the MS1 features with peptide and protein information. False discovery rate for the quantifiable proteins with at least two peptides was estimated to 1% using the target-decoy strategy (Kall et al., 2008). The four experimental conditions matured *in vivo* and matured *in vitro* (4x n=5) were compared with each other in a between subject design (Table 3). Only proteins with at least two identified peptides were evaluated in the statistical analysis. Differently expressed proteins were defined with a fold change >2 along with $p \leq 0.05$ (t-Test). String-database (http://string-db.org) was utilized for enrichment analysis of the differently expressed proteins (Szklarczyk et al., 2015). Up- and downregulated proteins were overlaid to all detected proteins in the experiment and analysed for overrepresentation of KEGG pathways.

From the other differently expressed proteins, a selection of interesting proteins was conducted according to their potential role in the *Cumulus oophorus* during maturation and in post-maturational functions. Significant enriched pathways as well as the selection of further interesting proteins are described in the result section. Significant differences between the groups for these proteins are illustrated in figures generated with PRISM7 (GraphPad Software, La Jolla, USA).

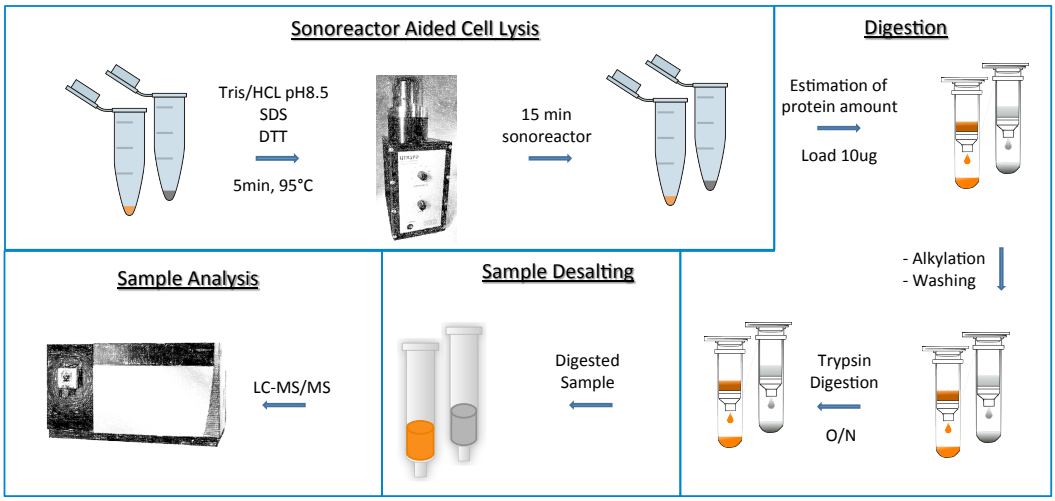

Figure 6: Sample preparation workflow: Samples are resuspended in lysis buffer and lysed by heating and high focused ultra-sonication. 10µg per sample were washed, reduced, alkylated and digested over night. After sample clean up the samples were measured by reverse phase LC-MS/MS operated in data dependent acquisition mode.

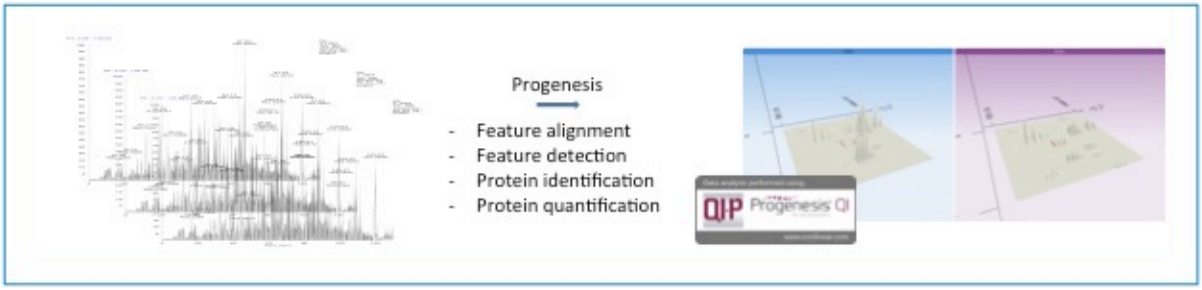

Figure 7: Data analysis overview: MS raw data were imported to Progenesis QI (Nonlinear Dynamics). Progenesis QI was used for MS feature detection, MS feature alignment, and after importing MASCOT search results the software performed relative protein quantification.

5.7 Collaboration with other Institutes and Units of the University of Zurich

The Functional Genomic Center Zurich (FGCZ) a core facility of the Swiss Federal Institute of Technology Zurich (ETH) and the University of Zurich (UZH) provided the proteomic analysis. Claudia Fortes and Dr. Bernd Roschitzki developed the method for the analysis of minute cumulus samples and supported the project through all stages. Dr. Jonas Grossmann guided the data analysis.

Permission animal experiment: a licence to perform animal experiments (241/2013) was delivered by the Cantonal Veterinary Office of Zurich.

6 Results

6.1 Proteome Analysis

In the 20 examined cumulus samples a total amount of 2277 proteins were quantifiable (≥ 2 peptides per protein). The protein false discovery rate (protFDR) was estimated using the target-decoy strategy and adjusted at 1% protein FDR (Kall et al., 2008). The four different biological groups were compared with each other to identify statistical relevant changes in protein abundance. The protein fold change (FC) cut-off value was set to a fold change of >2 in combination with $p<0.05$.

In cumulus samples from successfully matured COCs, 459 proteins were significantly differentially expressed between *in vitro* and *in vivo* successfully matured cumulus (complete significant results table 11.1). 308 proteins were upregulated in the *in vivo* group and 151 were upregulated in the *in vitro* group.

In cumulus samples of COCs that underwent *in vivo* maturation conditions, 360 proteins were significantly differentially expressed between the two maturation outcomes (complete significant results table 11.2). 240 of these proteins were upregulated in the COCs that matured successfully *in vivo* and the other 120 in the group that failed to mature *in vivo*.

In the cumulus samples from the COCs that failed to mature under both maturation conditions, 152 proteins were significantly differentially expressed after *in vitro* and *in vivo* maturation (complete significant results table 11.3). 63 proteins were upregulated in the COCs that failed to mature *in vivo* and 89 were upregulated in the group that failed to mature *in vitro*.

In the cumulus collected after *in vitro* maturation of the COCs, only 19 proteins were significantly differentially expressed between cumulus that matured successfully *in vitro* or that failed to mature (complete significant results table 11.4). Most upregulated proteins were in the group that matured successfully (n=13), compared to the COCs that failed to (n=6).

6.2 Enrichment analysis

The significantly different expressed proteins were assigned for an enrichment analysis for KEGG Pathways using String DB software (Franceschini et al., 2013). Significantly overrepresented KEGG pathways were detected in proteins upregulated in *in vivo* matured COCs compared to *in vitro* matured, as well as in proteins upregulated in *in vivo* matured COCs compared to *in vivo* failed to mature COCs. For the upregulated proteins of cumulus from COCs that either failed to mature *in vivo* and *in vitro* and the successfully matured *in vitro* and failed to mature *in vitro* groups no KEGG pathways were overrepresented.

6.2.1 Successfully matured cumulus: *in vitro* versus *in vivo*

From the 459 proteins significantly different expressed between the successfully matured cumulus *in vitro* and *in vivo,* 434 proteins were represented in the database.
Enrichment analysis for the upregulated proteins after successful *in vivo* maturation revealed the following overrepresented KEGG pathways:

- Complement and coagulation cascades (21 proteins, $p<0.0001$) (Table 4, Figures 8 & 9)
- Steroid biosynthesis (7 proteins, $p=0.0025$) (Table 5, Figures 10 & 11)
- N-Glycan biosynthesis (7 proteins, $p=0.04$) (Table 6, Figures 12 & 13)
- ECM-receptor interaction (11 proteins, $p=0.04$) (Table 7, Figures 14 &15)

For the *in vitro* matured group no enriched pathways were detected.

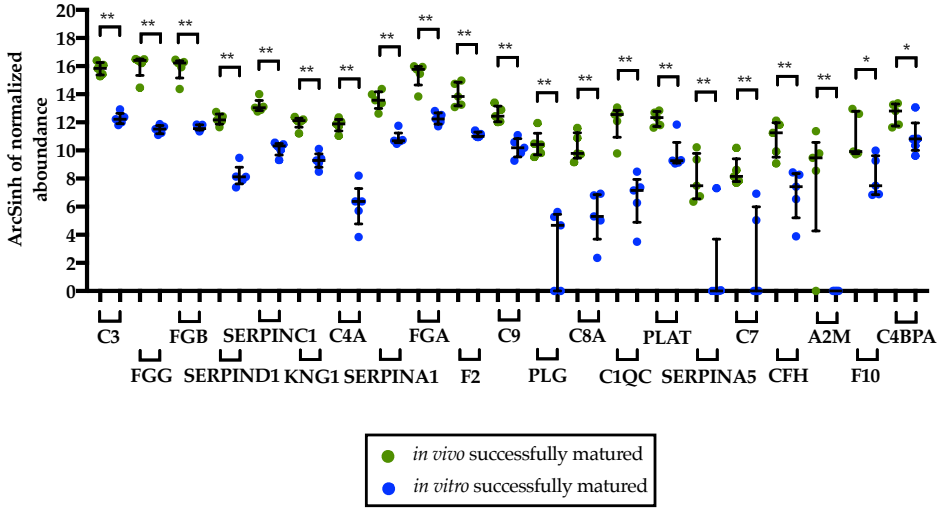

Figure 8: Significant upregulation of complement and coagulation cascade proteins in *in vivo* matured compared to *in vitro* matured cumulus ($p<0.05$, FC >2). Significant regulated proteins with $p<0.05$ are represented with one asterisk (*) and proteins significantly regulated with a p-value of $p<0.01$ with two asterisk (**).

Table 4: Twenty-one proteins involved in the complement and coagulation cascades with significant upregulation in the cumulus of *in vivo* successfully matured compared to *in vitro* successfully matured oocytes. The table illustrates the means and standard deviations (SD) of the ASINh transformed normalized protein abundances of the label-free quantification using Progenesis QI Software (Nonlinear Dynamics). The fold change (FC) was calculated on the non-transformed protein abundances, these data are shown in the complete significant results table 11.1.

KEGG pathway	Protein	Gene-ID	Uniprot-ID	Normalized mean *in vivo* successfully matured ±SD	Normalized mean *in vitro* successfully matured ±SD	Fold change	P-value
Complement and coagulation cascade (p-value= <0.001)	Complement system proteins						
	C1QC protein (Fragment)	C1QC	Q1RMH5	12.03±1.29	6.56±1.88	151	<0.001
	Complement C3	C3	Q2UVX4	15.82±0.46	12.26±0.42	35	<0.001
	Uncharacterized protein (complement C4-A)	C4A	E1BH06	11.82±0.49	6.1±1.57	143	<0.001
	C4b-binding protein alpha chain	C4BPA	Q28065	12.59±0.79	10.95±0.28	2	<0.05
	Complement component C7	C7	Q29RQ1	8.51±1	2.4±3.35	34	<0.01
	Uncharacterized protein (complement component C8 alpha chain)	C8A	F1MX87	10.25±0.99	5.28±1.85	91	<0.001
	Complement component C9	C9	Q3MHN2	12.57±0.59	10.19±0.7	10	<0.001
	Complement factor H	CFH	Q28085	10.85±1.29	6.91±1.85	39	<0.01
	Coagulation cascade proteins						
	Alpha-2-macroglobulin variant 23	A2M	K4JF16	7.84±4.5	0±0	Infinity	<0.01
	Prothrombin	F2	P00735	13.98±0.85	11.11±0.19	22	<0.001
	Coagulation factor X	F10	F00743	11.01±1.63	8.1±1.44	21	<0.05
	Fibrinogen alpha chain	FGA	P02672	15.4±0.89	12.27±0.42	26	<0.001
	Fibrinogen beta chain	FGB	F1MAV0	15.86±0.85	11.63±0.2	82	<0.001
	Fibrinogen gamma-B chain	FGG	F1MGU7	16.02±0.87	11.49±0.31	109	<0.001
	Kininogen-1	KNG1	P01044	12±0.45	9.28±0.58	14	<0.001
	Tissue-type plasminogen activator	PLAT	Q28198	12.26±0.53	9.73±1.19	6	<0.01
	Plasminogen	PLG	P06868	10.45±0.93	3.11±2.86	444	<0.001
	Alpha-1-antiproteinase	SERPINA1	P34955	13.59±0.66	10.86±0.51	15	<0.001
	Plasma serine protease inhibitor	SERPINA5	Q9N212	8.04±1.68	1.47±3.27	28	<0.01
	Antithrombin-III	SERPINC1	P41361	13.17±0.49	10.14±0.49	21	<0.001
	SERPIND1 protein	SERPIND1	A6QPP2	12.22±0.4	8.2±0.78	44	<0.001

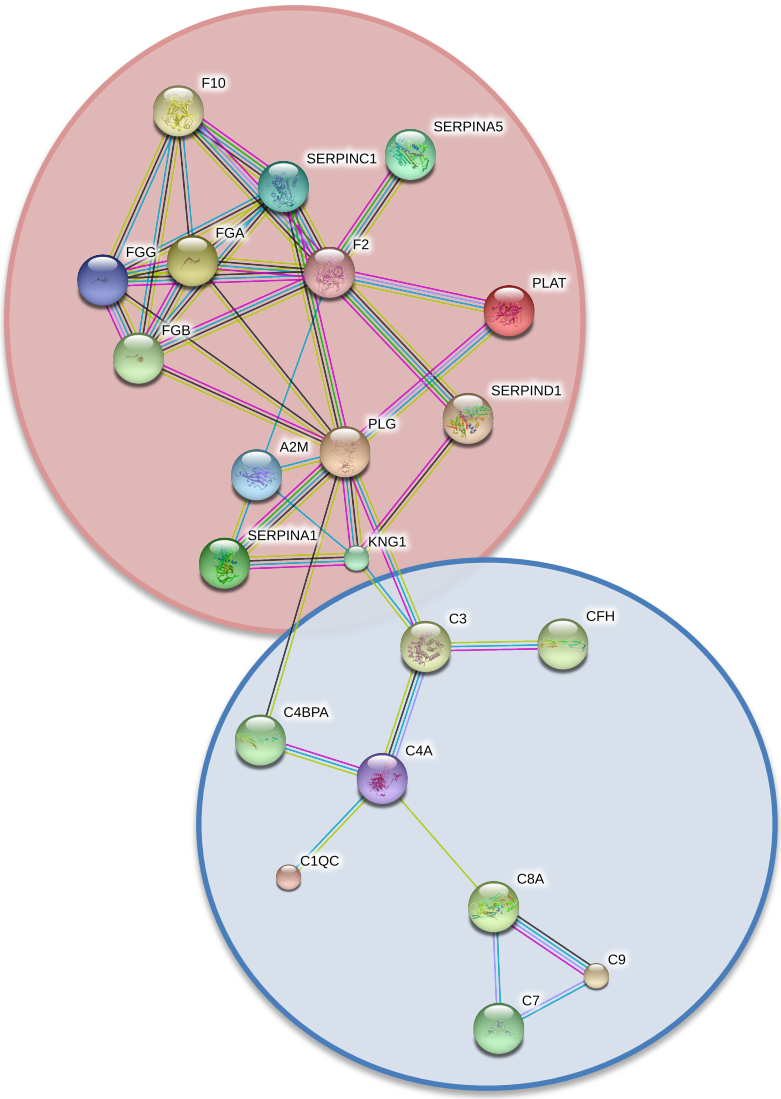

Figure 9: The twenty-one proteins involved in the complement and coagulation cascades and their interactions (interaction confidence: high (>0.7)). All these proteins were significantly upregulated ($p<0.05$, FC >2) in the cumulus of *in vivo* successfully matured COCs compared to *in vitro* matured as well as in the cumulus from *in vivo* successfully matured COCs compared to *in vivo* failed to mature.

The thirteen proteins in the red circle belong to the coagulation cascade: A2M, F2, F10, FGA, FGB, FGG, KNG1, PLAT, PLG, SERPINA1, SERPINA5, SERPINC1, SERPIND1. The eight proteins in the blue circle belong to the complement system: C1QC, C3, C4A, C4BPA, C7, C8A, C9 and CFH.

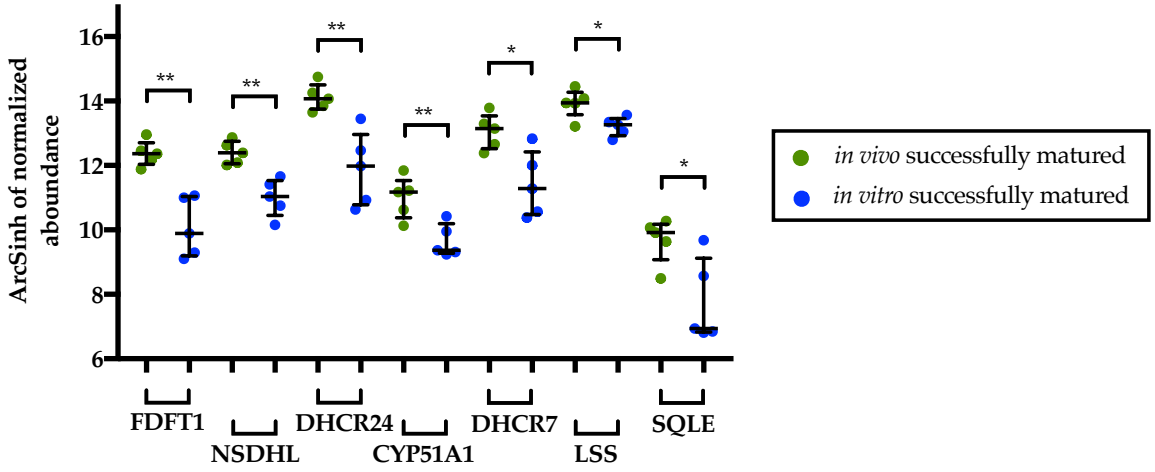

Figure 10: Significant upregulation of steroid biosynthesis proteins, in *in vivo* matured compared to *in vitro* matured cumulus ($p<0.05$, FC >2). Significant regulated proteins with $p<0.05$ are represented with one asterisk (*) and proteins significantly regulated with a p-value of $p<0.01$ with two asterisk (**).

Table 5: Seven proteins involved in steroid biosynthesis with significant upregulation in the cumulus from *in vivo* compared to *in vitro* successfully matured COCs. The table illustrates the means and standard deviations (SD) of the ASINh transformed normalized protein abundances of the label-free quantification using Progenesis QI Software (Nonlinear Dynamics). The fold change (FC) was calculated on the non-transformed protein abundances, these data are shown in the complete significant results table 11.1.

KEGG pathway	Protein	Gene-ID	Uniprot-ID	Normalized mean *in vivo* successfully matured ±SD	Normalized mean *in vitro* successfully matured ±SD	Fold change	P-value
Steroid biosynthesis (p-value= <0.01)	FDFT1 protein (squalene synthase)	FDFT1	Q6IE76	12.37±0.39	10.07±0.93	7	<0.001
	Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating	NSDHL	Q3ZBE9	12.4±0.36	11±0.59	3	<0.01
	DHCR24 protein	DHCR24	A6QR14	14.12±0.42	11.89±1.15	6	<0.01
	Lanosterol 14-alpha demethylase	CYP51A1	A6QR14	11±0.65	9.66±0.51	4	<0.01
	7-dehydrocholesterol reductase	DHCR7	G8JKY2	13.06±0.55	11.42±1.02	3	<0.05
	Lanosterol synthase	LSS	P84466	13.73±0.45	13.21±0.3	2	<0.05
	Squalene epoxidase	SQLE	A5D9A8	9.68±0.7	7.77±1.3	3	<0.05

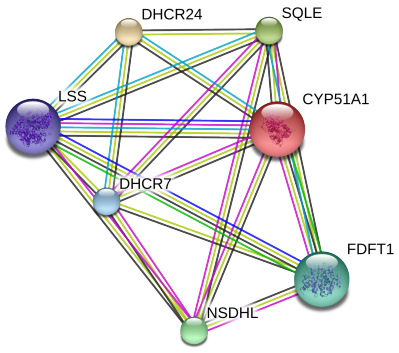

Figure 11: Interactions between the seven proteins significantly upregulated ($p<0.05$, FC >2) in the cumulus from *in vivo* compared to *in vitro* successful matured COCs involved in the steroid biosynthesis (interaction confidence: high (>0.7)).

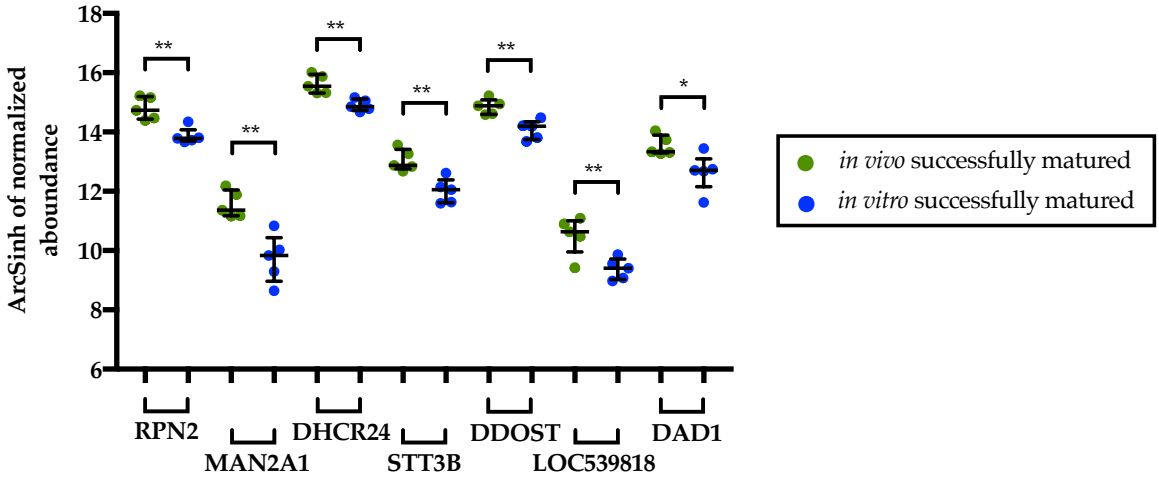

Figure 12: Significant upregulation of N-Glycan biosynthesis proteins, in *in vivo* matured compared to *in vitro* matured cumulus ($p<0.05$, FC >2). Significant regulated proteins with $p<0.05$ are represented with one asterisk (*) and proteins significantly regulated with a p-value of $p<0.01$ with two asterisk (**).

Table 6: Seven proteins significantly upregulated in the cumulus of *in vivo* successfully matured compared to *in vitro* successfully matured oocytes involved in N-Glycan biosynthesis. The table illustrates the means and standard deviations (SD) of the ASINh transformed normalized protein abundances of the label-free quantification using Progenesis QI Software (Nonlinear Dynamics). The fold change (FC) was calculated on the non-transformed protein abundances, these data are shown in the complete significant results table 11.1.

KEGG pathway	Protein	Gene-ID	Uniprot-ID	Normalized mean *in vivo* successfully matured ±SD	Normalized mean *in vitro* successfully matured ±SD	Fold change	P-value
N-Glycan biosynthesis (p-value= <0.05)	Dolichyl-diphosphooligo-saccharide-protein glycosyltransferase subunit 2	RPN2	Q3SZI6	14.8±0.38	13.87±0.27	2	<0.01
	Uncharacterized protein (alpha-mannosidase 2)	MAN2A1	F1N7T2	11.56±0.46	9.73±0.82	5	<0.01
	RPN1 protein	RPN1	A3KN04	15.62±0.32	14.9±0.2	2	<0.01
	STT3B protein	STT3B	A5D7G6	13.04±0.36	12.01±0.42	2	<0.01
	Dolichyl-diphosphooligo-saccharide-protein glycosyltransferase 48kDa subunit	DDOST	A6QPY0	14.85±0.26	14.08±0.32	2	<0.01
	Uncharacterized protein (Dolichyl-diphosphooligo-saccharide-protein glycosyltransferase subunit 1)	LOC539818	F1MJ36	10.51±0.65	9.38±0.36	3	<0.01
	Dolichyl-diphosphooligo-saccharide-protein glycosyltransferase subunit DAD1	DAD1	Q5E9C2	13.54±0.34	12.64±0.65	2	<0.05

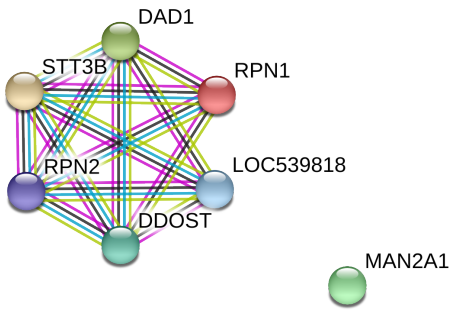

Figure 13: Interactions between the seven proteins significantly ($p<0.05$, FC >2) upregulated in the cumulus from *in vivo* successfully matured compared to *in vitro* successful matured oocytes involved in the N-Glycan biosynthesis (interaction confidence: high (>0.7)).

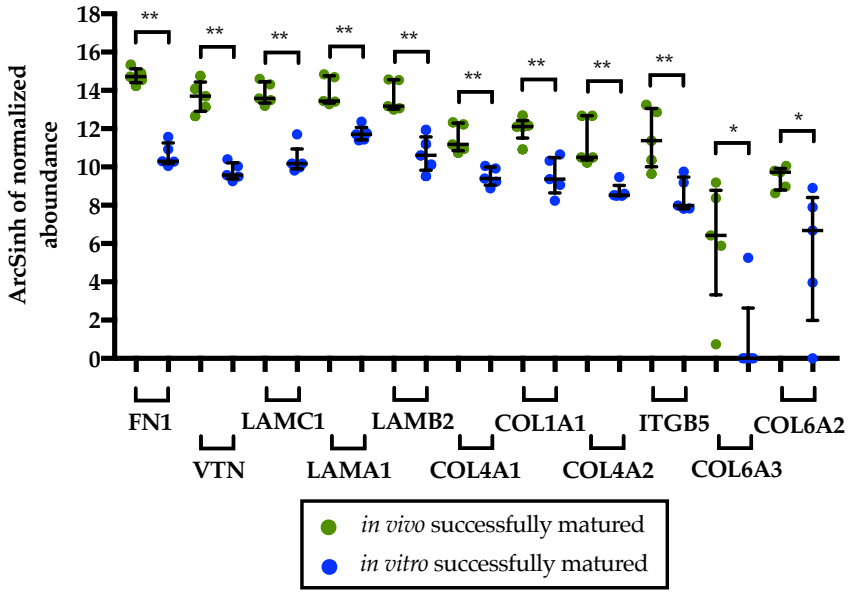

Figure 14: Significant upregulation of ECM-receptor interaction proteins in *in vivo* matured compared to *in vitro* matured cumulus ($p<0.05$, FC >2). Significant regulated proteins with $p<0.05$ are represented with one asterisk (*) and proteins significantly regulated with a p-value of $p<0.01$ with two asterisk (**).

Table 7: Eleven proteins significantly upregulated in the cumulus of successfully matured oocytes *in vivo* compared to *in vitro* involved in ECM-receptor interaction. The table illustrates the means and standard deviations (SD) of the ASINh transformed normalized protein abundances of the label-free quantification using Progenesis QI Software (Nonlinear Dynamics). The fold change (FC) was calculated on the non-transformed protein abundances, these data are shown in the complete significant results table 11.1.

KEGG pathway	Protein	Gene-ID	Uniprot-ID	Normalized mean *in vivo* successfully matured ±SD	Normalized mean *in vitro* successfully matured ±SD	Fold change	P-value
ECM-receptor interaction (p-value= <0.05)	Embryo-specific fibronectin 1 transcript variant	FN1	B8Y9S9	14.76±0.4	10.62±0.62	56	<0.001
	Uncharacterized protein (vitronectin)	VTN	Q3ZBS7	13.68±0.82	9.76±0.46	59	<0.001
	Uncharacterized protein (laminin subunit gamma-1)	LAMC1	F1MD77	13.84±0.59	10.37±0.76	27	<0.001
	Uncharacterized protein (laminin subunit alpha-1)	LAMA1	F1MEG3	13.94±0.76	11.74±0.39	10	<0.001
	Uncharacterized protein (laminin subunit beta-2)	LAMB2	E1BDK6	13.67±0.81	10.69±0.93	18	<0.001
	Collagen alpha-1 (IV) chain	COL4A1	G1K238	11.49±0.74	9.5±0.49	8	0.001
	Collagen alpha-1 (I) chain	COL1A1	P02453	12±0.65	9.53±0.98	9	<0.01
	Collagen alpha-2 (IV) chain (Fragment)	COL4A2	F1N7Q7	11.32±1.25	8.72±0.42	22	<0.01
	Integrin beta-5	ITGB5	P80747	11.51±1.55	8.51±0.9	31	<0.01
	Uncharacterized protein (collagen alpha-3 (VI) chain)	COL6A3	E1BB91	6.13±3.3	1.05±2.35	78	<0.05
	Uncharacterized protein (collagen alpha-2 (VI) chain)	COL6A2	F1MKG2	9.44±0.6	5.49±3.58	6	<0.05

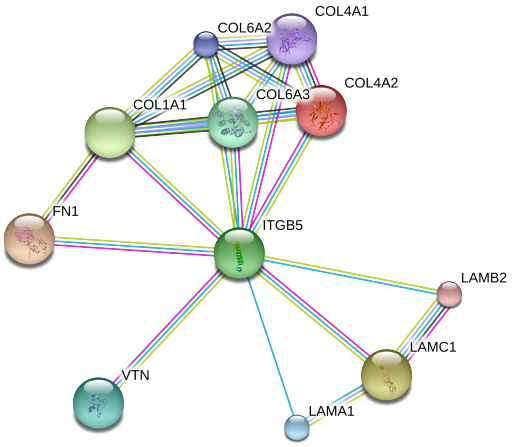

Figure 15: Interactions between the eleven proteins involved in ECM-receptor interaction with a significant ($p<0.05$, FC >2) higher expression in cumulus *in vivo* successfully matured compared to *in vitro* successfully matured oocytes (interaction confidence: high (>0.7)).

6.2.2 Maturation *in vivo*: Successfully matured versus COCs that failed to mature

From the 360 proteins significantly differentially expressed between cumulus matured successfully *in vivo* and cumulus from COCs that failed to mature, 341 proteins were recognized by the String software database and used for enrichment analysis.

Enrichment analysis for KEGG Pathways discovered three overrepresented pathways for the successfully matured group:

- Complement and coagulation cascades (21 proteins, $p<0.0001$) (Table 8, Figures 9 & 16)
- ECM-receptor interaction (11 proteins, $p=0.01$) (Table 9, Figures 17 & 18)
- Ovarian steroidogenesis (5 proteins, $p=0.058$) (Table 10, Figures 19 & 20)

For the cumulus that failed to mature *in vivo*, no enriched pathways were detected.

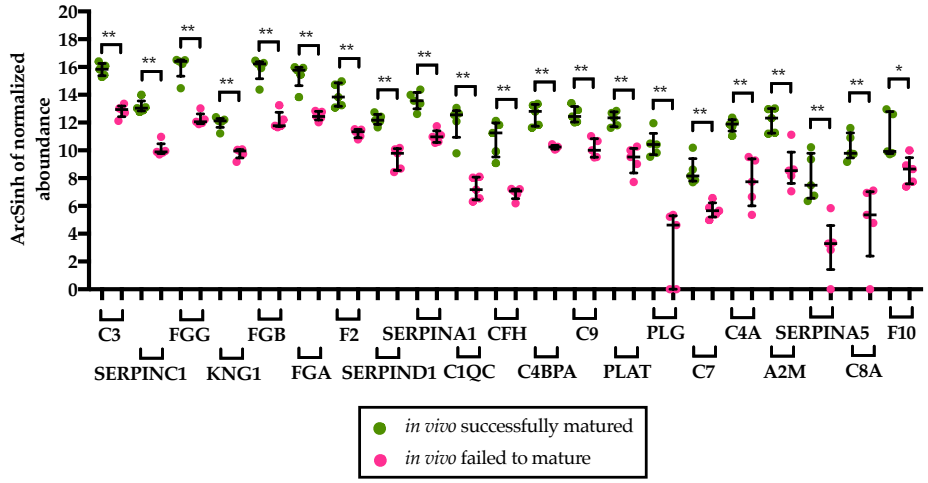

Figure 16: Significant upregulation of complement and coagulation proteins in cumulus of *in vivo* successfully matured COCs compared to *in vivo* failed to mature COCs ($p<0.05$, FC >2). Significant regulated proteins with $p<0.05$ are represented with one asterisk (*) and proteins significantly regulated with a p-value of $p<0.01$ with two asterisk (**).

Table 8: Twenty-one proteins involved in the complement and coagulation cascades present a significant higher expression in the cumulus from *in vivo* successfully matured COCs compared to the COCs that failed to mature *in vivo*. The table illustrates the means and standard deviations (SD) of the ASINh transformed normalized protein abundances of the label-free quantification using Progenesis QI Software (Nonlinear Dynamics). The fold change (FC) was calculated on the non-transformed protein abundances, these data are shown in the complete significant results table 11.2.

KEGG pathway	Protein	Gene-ID	Uniprot-ID	Normalized mean *in vivo* successfully matured ±SD	Normalized mean *in vivo* failed to mature ±SD	Fold change	P-value
Complement and coagulation cascade (p-value= <0.001)	Complement system proteins						
	C1QC protein (Fragment)	C1QC	Q1RMH5	12.03±1.16	7.24±0.82	140	<0.001
	Complement C3	C3	Q2UVX4	15.82±0.41	12.83±0.46	20	<0.001
	Uncharacterized protein (complement C4-A)	C4A	E1BH06	11.82±0.44	7.71±1.76	27	0.001
	C4b-binding protein alpha chain	C4BPA	Q28065	12.59±0.71	10.23±0.15	13	<0.001
	Complement component C7	C7	Q29RQ1	8.51±0.9	5.7±0.59	24	<0.001
	Uncharacterized protein (complement component C8 alpha chain)	C8A	F1MX87	10.25±0.88	4.84±2.88	81	<0.01
	Complement component C9	C9	Q3MHN2	12.57±0.52	10.14±0.69	11	<0.001
	Complement factor H	CFH	Q28085	10.85±1.16	6.91±0.43	81	<0.001
	Coagulation cascade proteins						
	Alpha 2-macroglobulin	A2M	Q7SIH1	12.16±0.79	8.7±1.49	15	<0.01
	Prothrombin	F2	P00735	13.98±0.76	11.24±0.31	20	<0.001
	Coagulation factor X	F10	P00743	11.01±1.46	8.55±1.02	20	<0.05
	Fibrinogen alpha chain	FGA	P02672	15.4±0.8	12.49±0.33	22	<0.001
	Fibrinogen beta chain	FGB	F1MAV0	15.86±0.76	12.14±0.66	41	<0.001
	Fibrinogen gamma-B chain	FGG	F1MGU7	16.02±0.78	12.23±0.46	49	<0.001
	Kininogen-1	KNG1	P01044	12±0.4	9.8±0.37	9	<0.001
	Tissue-type plasminogen activator	PLAT	Q28198	12.26±0.48	9.31±1.01	16	<0.001
	Plasminogen	PLG	P06868	10.45±0.83	3.04±2.79	518	<0.001
	Alpha-1-antiproteinase	SERPINA1	P34955	13.59±0.59	10.99±0.48	14	<0.001
	Plasma serine protease inhibitor	SERPINA5	Q9N212	8.04±1.5	3.07±2.08	102	<0.01
	Antithrombin-III	SERPINC1	P41361	13.17±0.44	10.08±0.51	22	<0.001
	SERPIND1 protein	SERPIND1	A6QPP2	12.22±0.36	9.43±0.81	14	<0.001

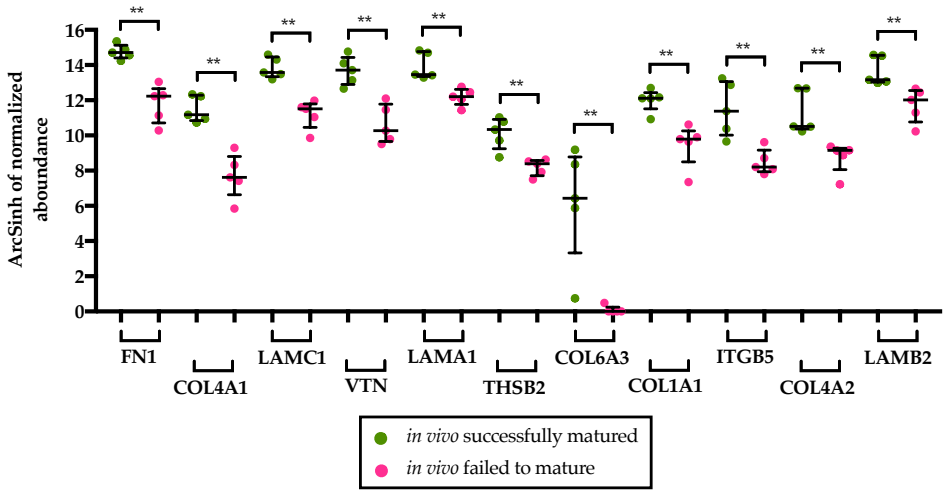

Figure 17: Significant upregulation of proteins involved in ECM-receptor interactions in *in vivo* successfully matured compared to *in vivo* failed to mature cumulus ($p<0.05$, FC >2). Significant regulated proteins with $p<0.05$ are represented with one asterisk (*) and proteins significantly regulated with a p-value of $p<0.01$ with two asterisk (**).

Table 9: Eleven proteins involved in ECM-receptor interaction are significantly overexpressed in the cumulus of COCs successfully matured *in vivo* compared to failed to mature *in vivo*. The table illustrates the means and standard deviations (SD) of the ASINh transformed normalized protein abundances of the label-free quantification using Progenesis QI Software (Nonlinear Dynamics). The fold change (FC) was calculated on the non-transformed protein abundances, these data are shown in the complete significant results table 11.2.

KEGG pathway	Protein	Gene-ID	Uniprot-ID	Normalized mean *in vivo* successfully matured ±SD	Normalized mean *in vivo* failed to mature ±SD	Fold change	P-value
ECM-receptor interaction (p-value= <0.05)	Embryo-specific fibronectin 1 transcript variant	FN1	B8Y9S9	14.76±0.36	11.8±1.08	14	<0.001
	Collagen alpha-1 (IV) chain	COL4A1	G1K238	11.49±0.67	7.7±1.27	32	<0.001
	Uncharacterized protein (laminin subunit gamma-1)	LAMC1	F1MD77	13.84±0.53	11.2±0.82	13	<0.001
	Uncharacterized protein (vitronectin)	VTN	Q3ZBS7	13.68±0.73	10.63±1.11	17	0.001
	Uncharacterized protein (laminin subunit alpha-1)	LAMA1	F1MEG3	13.94±0.68	12.19±0.49	7	<0.01
	Thrombospondin-2	THBS2	F1N1W3	10.13±0.82	8.19±0.48	8	<0.01
	Uncharacterized protein (collagen alpha-3 (VI) chain)	COL6A3	E1BB91	6.13±2.95	0.1±0.22	14883	<0.01
	Collagen alpha-1 (I) chain	COL1A1	P02453	12±0.58	9.46±1.24	10	<0.01
	Integrin beta-5	ITGB5	P80747	11.51±1.39	8.49±0.71	36	<0.01
	Collagen alpha-2 (IV) chain (Fragment)	COL4A2	F1N7Q7	11.32±1.11	8.76±0.87	19	<0.01
	Uncharacterized protein (laminin subunit gamma-1)	LAMB2	E1BDK6	13.67±0.73	11.73±0.99	7	<0.01

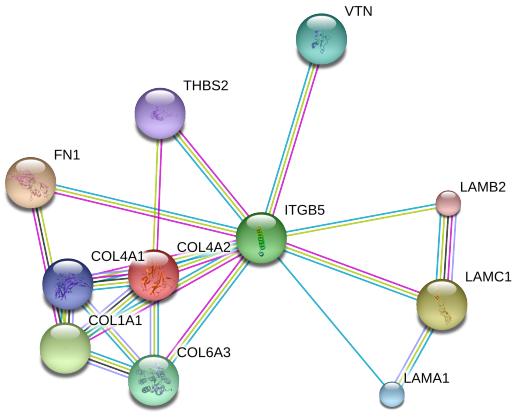

Figure 18: Interactions between the eleven proteins involved in ECM-receptor interaction with a significant ($p<0.05$, FC >2) higher expression in cumulus of *in vivo* successfully matured compared to *in vivo* failed to mature oocytes (interaction confidence: high (>0. 7)).

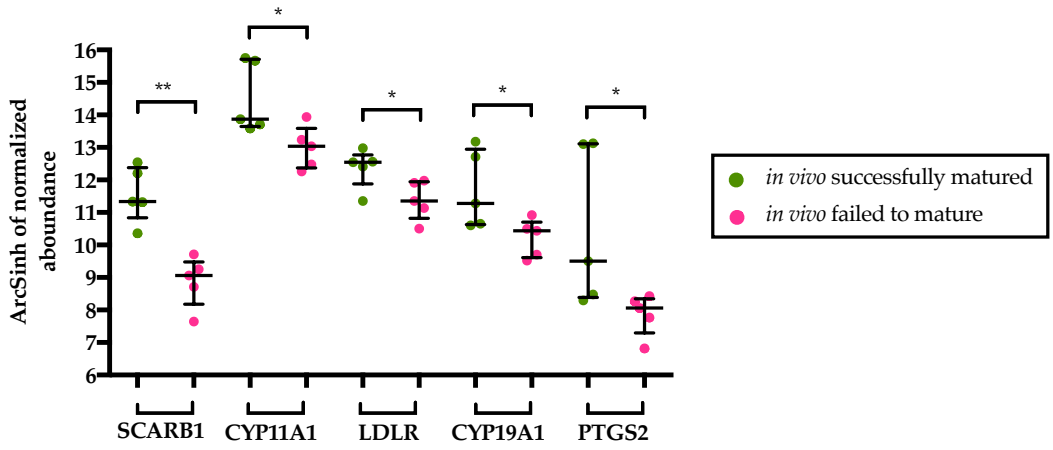

Figure 19: Significant upregulation of proteins involved in ovarian steroidogenesis in *in vivo* successfully matured compared to *in vivo* failed to mature cumulus ($p<0.05$, FC >2). Significant regulated proteins with $p<0.05$ are represented with one asterisk (*) and proteins significantly regulated with a p-value of $p<0.01$ with two asterisk (**).

Table 10: Five proteins involved in ovarian steroidogenesis show a significant higher expression in cumulus of *in vivo* successfully matured compared to *in vivo* failed to mature COCs. The table illustrates the means and standard deviations (SD) of the ASINh transformed normalized protein abundances of the label-free quantification using Progenesis QI Software (Nonlinear Dynamics). The fold change (FC) was calculated on the non-transformed protein abundances, these data are shown in the complete significant results table 11.2.

KEGG pathway	Protein	Gene-ID	Uniprot-ID	Normalized mean *in vivo* successfully matured ±SD	Normalized mean *in vivo* failed to mature ±SD	Fold change	P-value
Ovarian steroido-genesis (p-value= 0.058)	Scavenger receptor class B member 1	SCARB1	O18824	11.56±0.77	8.88±0.78	16	<0.001
	Cholesterol side-chain cleavage enzyme, mitochondrial	CYP11A1	P00189	14.52±0.98	12.99±0.66	6	<0.05
	Low-density lipoprotein receptor	LDLR	F1MZ58	12.37±0.54	11.38±0.61	3	<0.05
	Aromatase	CYP19A1	P46194	11.69±1.07	10.22±0.58	7	<0.05
	Prostaglandin G/H synthase 2	PTGS2	F1MNI5	10.5±2.17	7.87±0.64	68	<0.05

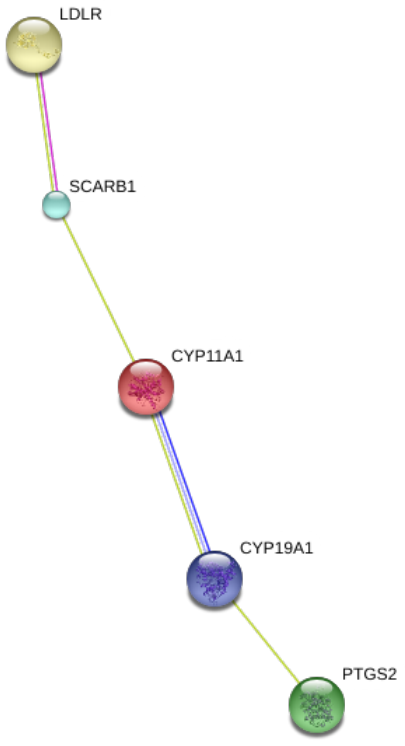

Figure 20: Interactions between the five proteins involved in ovarian steroidogenesis with a significant ($p<0.05$, FC >2) higher expression in cumulus from *in vivo* successfully matured compared to *in vivo* failed to mature COCs (interaction confidence: high (>0.7)).

6.3 Other proteins of biological interest with significant differences

Beneath the proteins of the overrepresented pathways presented in the previous chapter, numerous proteins that are highly interesting for oocyte maturation were in the groups of significantly differentially expressed proteins. A selection of these individual proteins was grouped according to their potential biological function and will be presented in the following chapter (Table 11).

Table 11: Individual proteins selected for interesting biological function, manually grouped and listed by these functions:

The table illustrates the means and standard deviations (SD) of the ASINh transformed normalized protein abundances of the label-free quantification using Progenesis QI Software (Nonlinear Dynamics). The fold change (FC) was calculated on the non-transformed protein abundances, these data are shown in the complete significant results tables 11.1 – 11.4.

Significant different expression between the maturation conditions and outcomes (SM=successfully matured; FM= failed to mature) are summarized with fold change (FC) and p-value of the t-Test.

Protein	Gene-ID	UniProt-ID	Normalized mean *in vivo* successfully matured ±SD	Normalized mean *in vivo* failed to mature ±SD	Normalized mean *in vitro* successfully matured ±SD	Normalized mean *in vitro* failed to mature ±SD	Significant differences between groups, with Fold change (FC)
Oxidative stress defence							
Cystatin-B	**CSTB**	P25417	12.29±0.52	11.64±0.85	12.30±0.57	11.39±0.33	*in vitro* SM > *in vitro* FM (FC 3; p<0.05)
Thioredoxin reductase 2, mitochondrial	**TXNRD2**	Q9N8I8	5.24±3.18	9.13±1.12	9.96±0.36	8.82±0.72	*in vitro* SM > *in vitro* FM (FC 3; p<0.05)
Copper transport protein	**ATOX1**	Q3T0E0	11.61±0.23	12.95±0.39	12.68±0.25	12.09±0.35	*in vivo* SM < *in vivo* FM (FC 4; p<0.01) *in vivo* SM < *in vitro* SM (FC 3; p<0.01)
Peroxiredoxin-6	**PRDX6**	O77834	14.34±0.45	15±0.46	15.79±0.28	15.22±0.4	*in vivo* SM < *in vitro* SM (FC 4; p<0.01)
Versican core protein	**VCAN**	P81282	13.48±0.45	11.15±0.24	11.37±0.55	11.67±0.89	*in vivo* SM > *in vivo* FM (FC 11; p<0.01) *in vivo* SM > *in vitro* SM (FC 8; p<0.01)
Caveolin-1	**CAV1**	P79132	11.43±1.13	7.4±0.48	7.77±0.64	7.94±1.03	*in vivo* SM > *in vivo* FM (FC 84; p<0.01) *in vivo* SM > *in vitro* SM (FC 54; p<0.01)
Antithrombin-III	**SERPINC1**	P41361	13.17±0.49	10.08±0.51	10.14±0.49	10.79±0.88	*in vivo* SM > *in vivo* FM (FC 22; p<0.01) *in vivo* SM > *in vitro* SM (FC 21; p<0.01)
Serotransferrin	**TF**	G3X6N3	15.16±0.6	12.45±0.82	11.92±1.15	11.81±0.49	*in vivo* SM > *in vivo* FM (FC 14; p<0.01) *in vivo* SM > *in vitro* SM (FC 16; p<0.01)
Superoxide-dismuthase (Cu-Zn)	**SOD1**	P00442	13.48±0.7	12.64±0.79	12.59±0.24	12.5±0.63	*in vivo* SM > *in vitro* SM (FC 3; p<0.05)
Modulation of apoptosis							
Caspase-3	**CASP3**	Q08DY9	9.71±0.99	10.92±0.33	11.08±0.18	10.71±0.56	*in vivo* SM < *in vivo* FM (FC 3; p<0.05) *in vivo* SM < *in vitro* SM (FC 3; p<0.05)
Repair of DNA damage							
DNA-(apurinic or apyrimidinic site) lyase	**APEX1**	P23196	13.68±0.4	14.2±0.42	14.47±0.13	14.13±0.3	*in vivo* SM < *in vitro* SM (FC 2; p<0.01)
Gas transport							
Hemoglobin subunit alpha	**HBA**	P01966	16.97±1.1	13.08±1.38	12.47±0.33	12.52±0.7	*in vivo* SM > *in vivo* FM (FC 26; p<0.01) *in vivo* SM > *in vitro* SM (FC 123; p<0.01)
Protein Group: Hemoglobin subunit beta	**HBB**	P02070	13.63±1.94	10.42±0.89	9.2±0.94	12.55±0.46	*in vivo* SM > *in vivo* FM (FC 6; p<0.05) *in vivo* SM > *in vitro* SM (FC 21; p<0.01)
	HBB	D4QBB3	14.95±0.89	13.12±0.87	12.04±0.75	9.05±0.8	*in vivo* SM > *in vivo* FM (FC 43; p<0.01) *in vivo* SM > *in vitro* SM (FC 145; p<0.01)
Stability and expansion of cumulus							
Inter-alpha-trypsin inhibitory heavy chain H1	**ITIH1**	Q0VCM5	12.94±2.38	9.34±0.79	9.35±0.46	9.14±0.68	*in vivo* SM > *in vivo* FM (FC 100; p<0.05) *in vivo* SM > *in vitro* SM (FC 111; p<0.05)
Inter-alpha-trypsin inhibitory heavy chain H2	**ITIH2**	F1MNW4	17.82±0.5	12.97±0.21	12.86±0.23	12.96±0.32	*in vivo* SM > *in vivo* FM (FC 139; p<0.01) *in vivo* SM > *in vitro* SM (FC 154; p<0.01)
Inter-alpha-trypsin inhibitory heavy chain H3	**ITIH3**	P56652	14.56±0.53	10.21±0.86	9.93±1.34	10.59±1.37	*in vivo* SM > *in vivo* FM (FC 68; p<0.01) *in vivo* SM > *in vitro* SM (FC 63; p<0.01)
Inter-alpha-trypsin inhibitory heavy chain H4	**ITIH4**	Q3T052	12.04±0.5	7.84±2.03	7.11±1.21	6.62±1.69	*in vivo* SM > *in vivo* FM (FC 20; p<0.01) *in vivo* SM > *in vitro* SM (FC 82; p<0.01)
Pentraxin-related protein	**PTX3**	Q0VCG9	12.95±1.51	10.29±0.57	10.63±0.28	10.65±0.53	*in vivo* SM > *in vivo* FM (FC 29; p<0.01) *in vivo* SM > *in vitro* SM (FC 22; p<0.01)
Tumor necrosis factor alpha induced protein 6	**tsg-6**	Q5W1C4	16.65±0.55	12.94±0.15	12.95±0.13	12.93±0.28	*in vivo* SM > *in vivo* FM (FC 46; p<0.01) *in vivo* SM > *in vitro* SM (FC 46; p<0.01)
Prostaglandin G/H synthase 2	**PTGS2**	F1MNI5	10.5±2.43	7.87±0.64	7.62±0.58	7.78±0.42	*in vivo* SM > *in vivo* FM (FC 68; p<0.05) *in vivo* SM > *in vitro* SM (FC 86; p<0.05)
Post-ovulatory processes							
CD9 antigen	**CD9**	P30932	11.64±0.74	9.97±0.51	11.75±0.59	11.7±0.43	*in vivo* SM > *in vivo* FM (FC 6; p<0.01)
Embryo-specific fibronectin 1 transcript variant	**FN1**	B8Y9S9	14.76±0.41	11.80±1.08	10.63±0.62	10.92±0.56	*in vivo* SM > *in vivo* FM (FC 14; p<0.01) *in vivo* SM > *in vitro* SM (FC 56; p<0.01)
Alpha-2-macroglobulin	**A2M**	Q7SIH1	12.16±0.88	8.71±1.49	8.79±1.49	8.38±1.14	*in vivo* SM > *in vivo* FM (FC 15; p<0.01) *in vivo* SM > *in vitro* SM (FC 20; p<0.01)
Influence on sperm							
Complement C3	**C3**	Q2UVX4	15.82±0.46	12.83±0.46	12.26±0.42	12.31±0.3	*in vivo* SM > *in vivo* FM (FC 20; p<0.01) *in vivo* SM > *in vitro* SM (FC 35; p<0.01)

Proteins involved in oxidative stress defence:

Regarding biological functions, nine proteins involved in oxidative stress defence were sorted with different expression between the groups and with significant differences between the groups; this different expression is to find in Figure 21 and Table 11.

Regarding both outcomes post *in vitro* maturation, two proteins were significantly overexpressed in cumulus that matured *in vitro* and cumulus that failed to mature *in vitro* ($p<0.05$, FC 3 resp 3): cystatin-B (CSTB) and mitochondrial thioredoxin reductase 2 (TXNRD2).

After *in vivo* maturation, only the copper transport protein ATOX1 was significantly overexpressed in *in vivo* failed to mature cumulus compared to *in vivo* matured ($p<0.01$, FC 4). In *in vivo* matured cumulus compared to *in vivo* failed to mature, four proteins present significant overexpression: versican core protein (VCAN), caveolin-1 (CAV1), antithrombin-III (SERPINC1) and serotransferrin (TF) ($p<0.01$, FC 11 resp. 84, 22, 14).

Regarding cumulus that matured with success under different maturation conditions, two proteins were significantly overexpressed in *in vitro* matured cumulus compared to *in vivo* matured ($p<0.01$, FC 3 resp 4): copper transport protein ATOX1 and peroxiredoxin-6 (PRDX6).

In *in vivo* matured compared to *in vitro* matured cumulus samples, five proteins were significant overexpressed: versican core protein, caveolin-1, antithrombin-III, serotransferrin ($p<0.01$, FC 8 resp. 54, 21, 16) and superoxide dismutase (Cu-Zn) (SOD1) ($p<0.05$, FC 3)

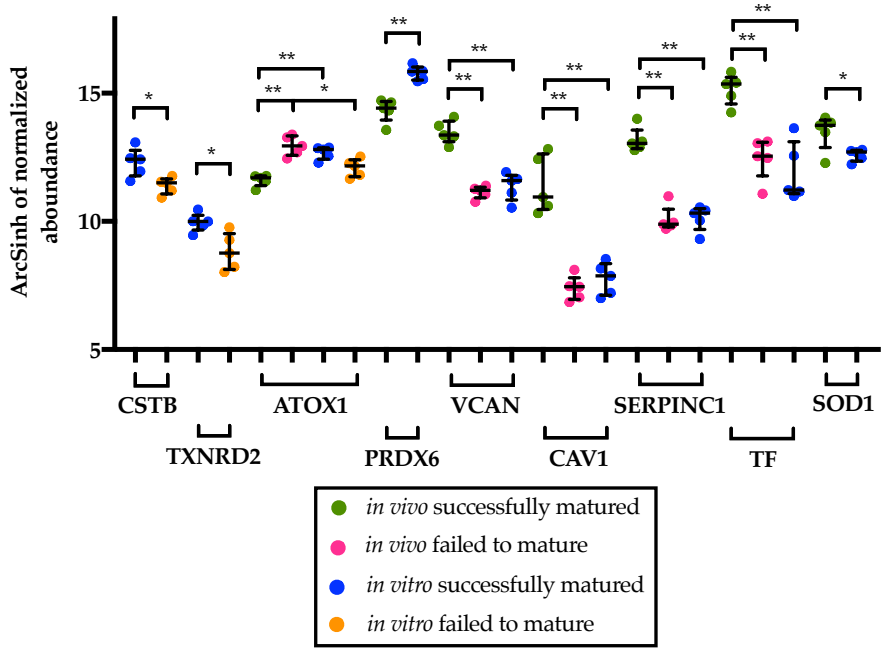

Figure 21: Expression of cystatin-B (CSTB), mitochondrial thioredoxin reductase 2 (TXNRD2), copper transport protein ATOX1, peroxiredoxin-6 (PRDX6), versican core protein (VCAN), caveolin-1 (CAV1), antithrombin-III (SERPINC1), serotransferrin (TF) and superoxide dismutase (Cu-Zn) (SOD1), proteins active in oxidative stress defence, for the statistically significant group comparisons. Significant regulated proteins with $p<0.05$ are represented with one asterisk (*) and proteins significantly regulated with a p-value of $p<0.01$ with two asterisk (**).

Caspase-3 as main protein of apoptotic pathways:

Caspase-3, involved in apoptosis modulation, was found in different groups, with significant differentially expression between the groups (Figure 22, Table 11).

Regarding *in vivo* maturation conditions, a significant overexpression was found in cumulus that failed to mature under *in vivo* conditions compared to cumulus that successfully matured *in vivo* ($p<0.05$, FC 3).

Between both maturation conditions, significantly more caspase-3 was expressed in *in vitro* matured cumulus compared to *in vivo* matured cumulus ($p<0.05$, FC 3).

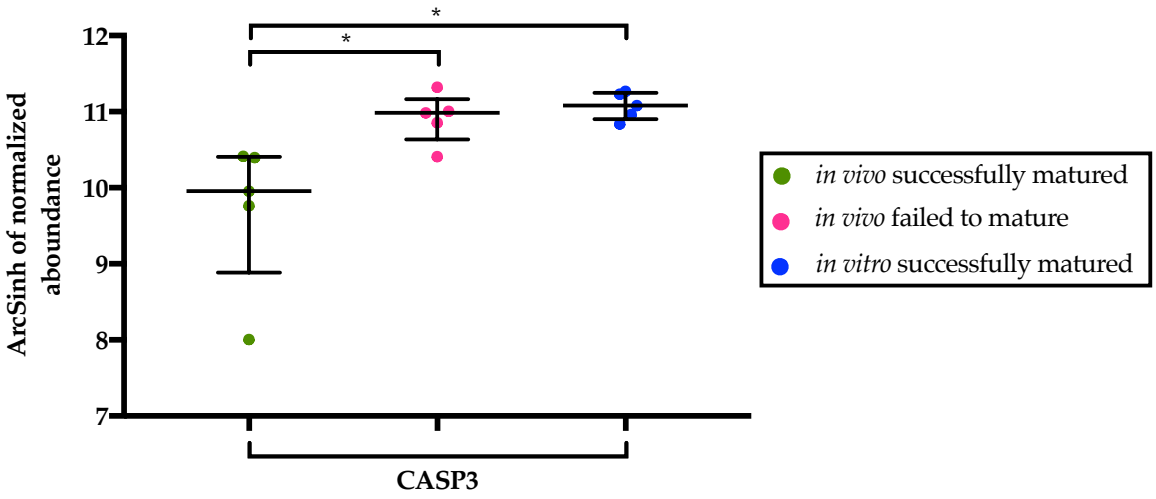

Figure 22: Expression of the protein Caspase-3 (CASP3), active in modulation of apoptosis, for the groups with significant differences. Significant regulated proteins with $p<0.05$ are represented with one asterisk (*).

APEX1, a protein involved in DNA repair:

APEX1, a protein with DNA repair function, was found significantly differentially expressed between both maturation conditions (Figure 23, Table 11).

In cumulus that matured under *in vitro* conditions, significantly more APEX1 was expressed compared to cumulus that matured *in vivo* ($p<0.01$, FC 2).

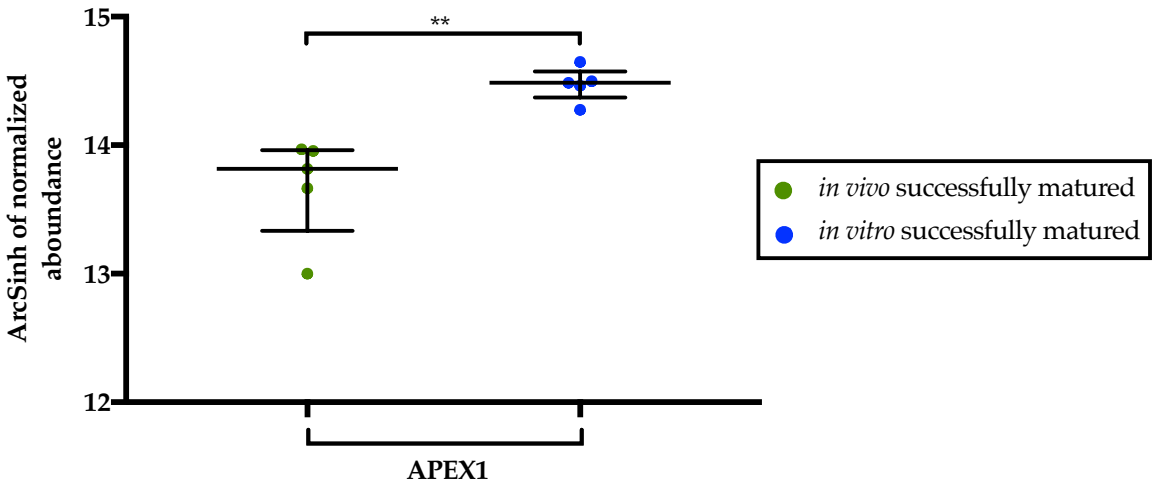

Figure 23: Expression of DNA-(apurinic or apyrimidinic site) lyase (APEX1), a protein active in repair of DNA damage, for the two maturation conditions with a statistical significant difference. Significant regulated proteins with a p-value of $p<0.01$ are represented here with two asterisk (**).

Hemoglobin subunits A and B as proteins involved in gas transport:

Hemoglobin subunit A and B, both involved in gas transport, were found with significant different expression levels between the groups (Figure 24, Table 11).

In *in vivo* matured cumulus samples, a significant higher expression of the proteins hemoglobin subunit alpha (HBA) ($p<0.01$, FC 26) and haemoglobin subunit beta (Protein Group HBB: Uniprot Accession P02070, $p<0.05$, FC 6; Uniprot Accession D4QBB3, $p<0.01$, FC 43) was found in COCs that matured with success compared to COCs that failed to mature. Between both maturation conditions, the proteins hemoglobin subunit alpha (HBA, $p<0.01$, FC 123) and the protein group for hemoglobin subunit beta with it's two variants (Protein Group HBB: P02070, $p<0.01$, FC 21; D4QBB3, $p<0.01$, FC 145), were overexpressed in cumulus from in *in vivo* matured COCs compared to *in vitro* matured COCs.

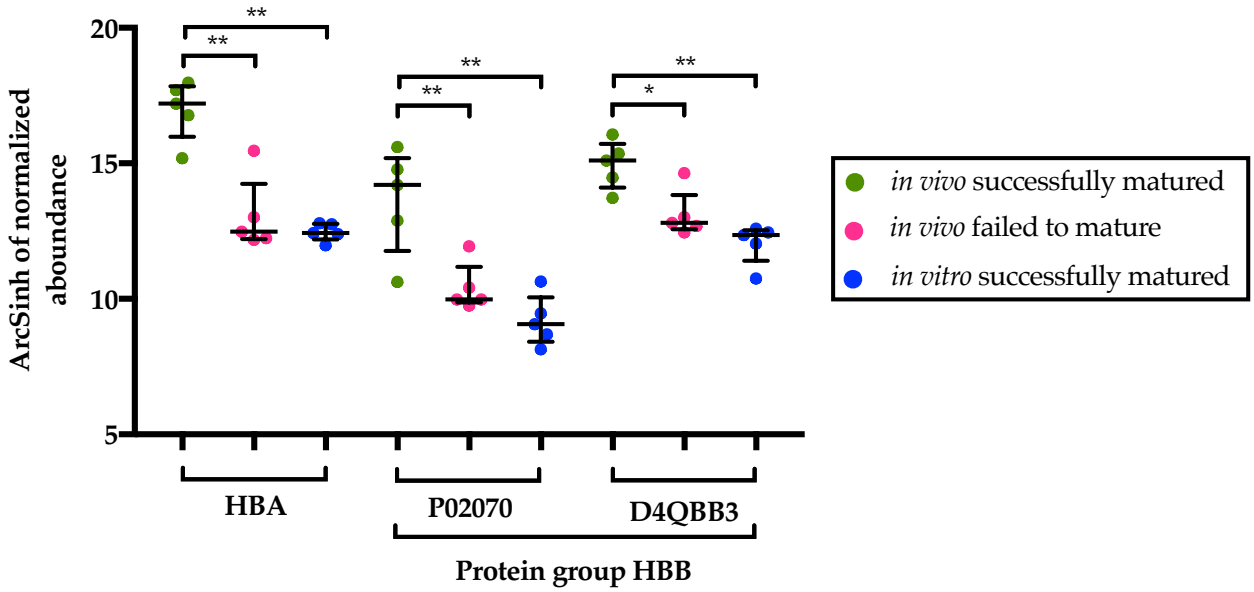

Figure 24: Expression of hemoglobin subunit alpha (HBA) and both variant of hemoglobin subunit beta (HBB) group, proteins active in gas transport, for the significant different groups. Significant regulated proteins with $p<0.05$ are represented with one asterisk (*) and proteins significantly regulated with a p-value of $p<0.01$ with two asterisk (**).

Proteins involved in stability and expansion of the *Cumulus oophorus*:

In the different groups, proteins involved in stability and expansion of the cumulus mass around oocyte were found, with significant different expression between the groups (Figure 25, Table 11).

After *in vivo* maturation, in successfully matured cumulus proteins like inter-alpha-trypsin inhibitor heavy chain H1 (ITIH1, Q0VCM5) ($p<0.05$, FC 100), inter-alpha-trypsin inhibitor heavy chain H2 (ITIH2), inter-alpha-trypsin inhibitor heavy chain H3 (ITIH3), and inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) ($p<0.01$, FC 139, 68 and 20) were overexpressed compared to cumulus from COCs that failed to mature *in vivo*.

Regarding the different maturation conditions, a significant overexpression of the same proteins inter-alpha-trypsin inhibitor heavy chain H1 (ITIH1) ($p<0.05$, FC 112), inter-alpha-trypsin inhibitor heavy chain H2 (ITIH2), inter-alpha-trypsin inhibitor heavy chain H3 (ITIH3), inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) ($p<0.01$, FC 154, 63 and 82) in *in vivo* matured cumulus compared to *in vitro* matured cumulus was found.

Parallel to proteins of the ITIH family, also pentraxin-related protein PTX3 ($p<0.01$, FC 29), tumor necrosis factor alpha induced protein 6 (tsg-6) ($p<0.01$, FC 46) and prostaglandin G/H

synthase 2 (PTGS2) ($p<0.05$, FC 68) were significantly overexpressed in *in vivo* successfully matured cumulus compared to *in vivo* failed to mature.

Also between maturation conditions, pentraxin-related protein PTX3 ($p<0.01$, FC 22), tumor necrosis factor alpha induced protein 6 (tsg-6) ($p<0.01$, FC 46) and prostaglandin G/H synthase 2 (PTGS2) ($p<0.05$, FC 86) were overexpressed in *in vivo* successfully matured compared to *in vitro* successfully matured.

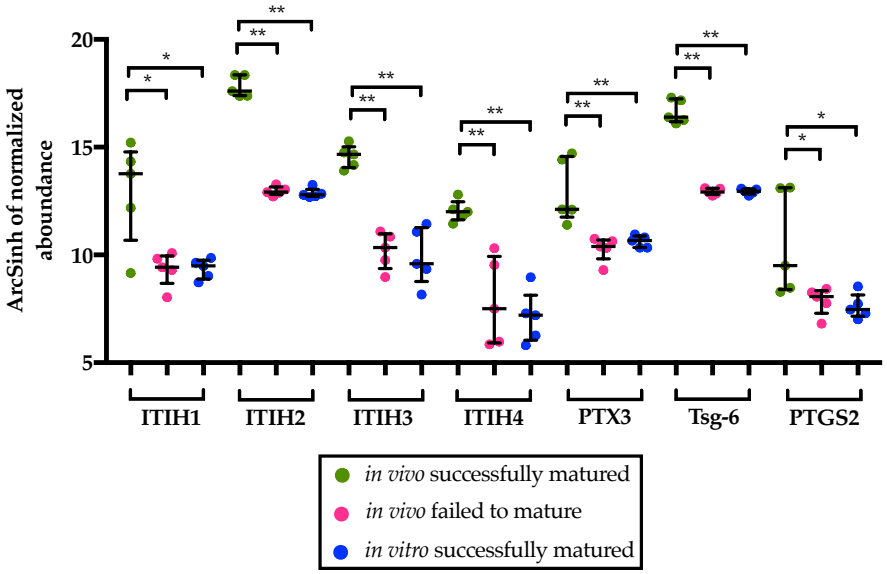

Figure 25: Expression of inter-alpha-trypsin inhibitor heavy chain H1 to H4 (ITIH1-4), pentraxin-related protein PTX3, tumor necrosis factor alpha induced protein 6 (tsg-6) and prostaglandin G/H synthase 2 (PTGS2). These proteins, active in stability and expansion of cumulus, present a significant different expression in the different groups. Significant regulated proteins with $p<0.05$ are represented with one asterisk (*) and proteins significantly regulated with a p-value of $p<0.01$ with two asterisk (**).

Proteins involved in post-ovulatory processes:

Three proteins involved in post-ovulatory events were found in the different groups with significant differently expression between the groups (Figure 26, Table 11).

After *in vivo* maturation, CD9 antigen (CD9), embryo-specific fibronectin 1 transcript variant (FN1) and alpha-2-macroglobulin (A2M) were significantly overexpressed in *in vivo* matured cumulus compared to *in vivo* failed to mature ($p<0.01$, FC 6 resp. 14 and 15).

Regarding maturation conditions, two proteins were significantly overexpressed in *in vivo* matured compared to *in vitro* matured: embryo-specific fibronectin 1 transcript variant and alpha-2-macroglobulin ($p<0.01$, FC 56 resp. 20).

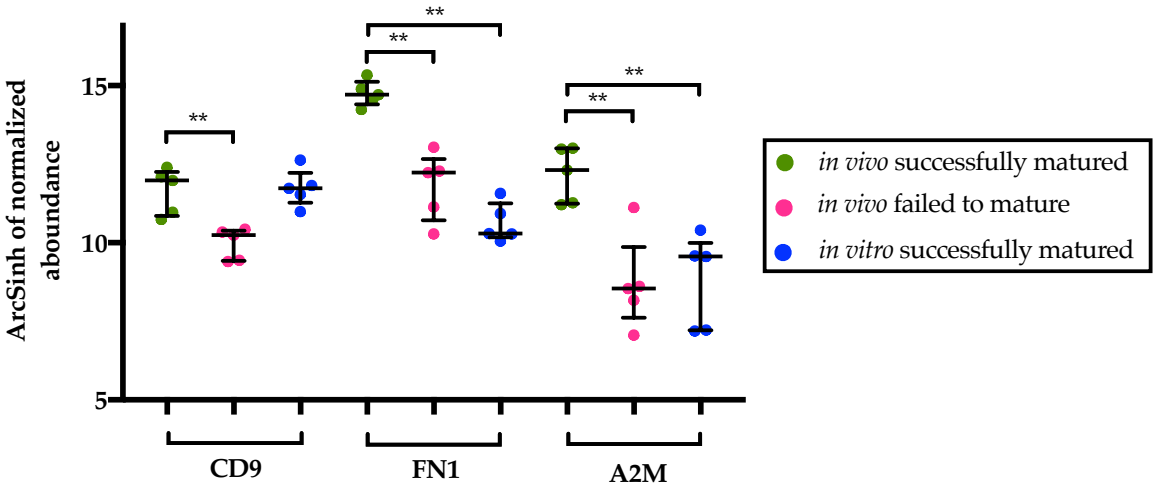

Figure 26: Expression of CD9 antigen (CD9), embryo-specific fibronectin 1 transcript variant (FN1) and alpha-2-macroglobulin (A2M), proteins active in post-ovulatory processes, for the groups with significant differences. Significant regulated proteins with p<0.01 are represented here with two asterisk (**).

Proteins with influence on sperm:

The protein complement C3 (C3), influencing sperm direction and motility was found in different groups with significant overexpression in *in vivo* matured cumulus compared to *in vivo* failed to mature (p<0.01, FC 20) and between *in vivo* matured and *in vitro* matured (p<0.01, FC 35) (Figure 27, Table 11).

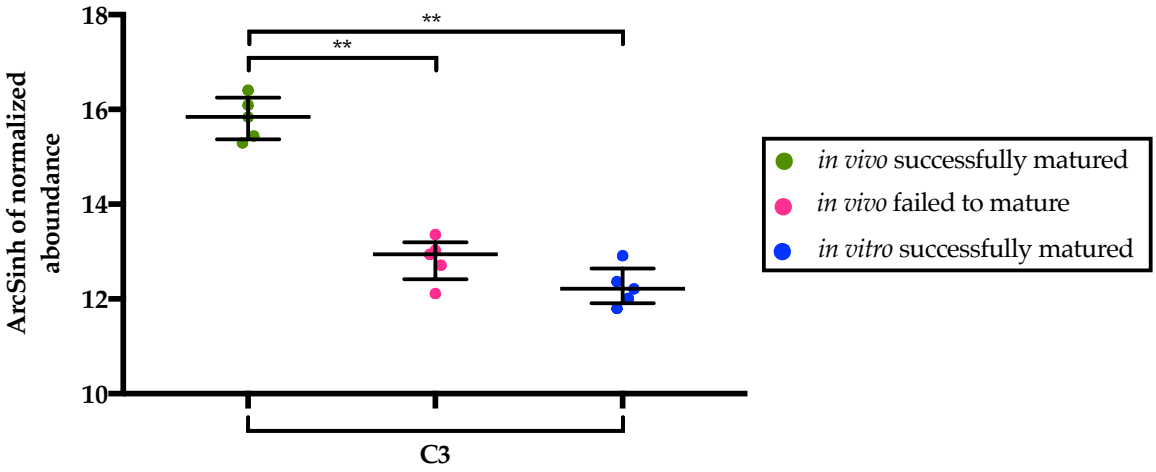

Figure 27: Expression of complement component C3 (C3), a protein influencing sperm, in the three groups with significant differences. Significant regulated proteins with p<0.01 are represented with two asterisk (**).

7 Discussion

7.1 Study design

The aim of this study was the investigation of the cumulus proteome of cumulus oocytes complexes matured under *in vitro* and *in vivo* conditions. Cumulus from superovulated heifers after induction of ovulation (*in vivo* group) was compared to cumulus from control animals without follicular stimulation for maturation *in vitro*. Cumulus from successfully matured oocytes as well as cumulus from oocytes that failed to mature was examined.

Cumulus samples were collected from a homogeneous group of six young, cycling Brown Swiss heifers from similar geographical regions and raising condition. This selection of a homogenous group of oocyte donors was performed to reduce the impact of COC origin on proteomic results as much as possible. Available literature in mammals shows a different protein expression in COCs depending on donor age (Al-Edani et al., 2014; Gandolfi et al., 1998; McReynolds et al., 2011; McReynolds et al., 2012). For this study, impact of age, breed and donor condition was reduced as much as possible with the donor selection.
Oocytes collected from slaughtered cows present a highly variable quality with a representation of all stages of oestrus cycle (Lonergan et al., 1994; Paczkowski and Krisher, 2010). The most homogenous group of COCs is usually recovered by ovum pick-up, by collection of synchronous and defined stages of follicles (Merton et al., 2012). Therefore, aspiration of dominant follicles in all six heifers was performed to allow a better superovulation response and obtain synchronized cycles to collect a homogenous population of COCs.

Duration of maturation after COC collection has also to be considered as influencing factor: the time in maturation medium is a more limiting factor for IVP success in COCs from slaughterhouse animals than from OPU animals (Merton et al., 2012). A prolonged maturation time impacts the further developmental competence of the oocyte (Nakagawa et al., 1995; Ocana-Quero et al.). For *in vitro* maturation of COCs from slaughterhouse origin, the development potential is at it's maximum between 18 and 24 hours IVM (Merton et al., 2012). Most of these bovine oocytes are in metaphase II after 20 hours (Agung et al., 2006). Development potential of OPU derived oocytes stays similar between 16 and 28 hours IVM

(Merton et al., 2012). In the present study, COCs of the *in vitro* maturation group were matured 21 hours *in vitro*.

For practical reasons, the *in vivo* matured COCs were collected from superovulated heifers, not on naturally cycling animals. This allowed the collection of a sufficient number of successfully matured COCs from only 3 heifers. The alternative approach: using repeated OPU sessions of single follicles was rejected due to the necessity to collect a sufficient amount of cumulus cells for proteomic analysis on single COC level. Studies on the effect of superovulation treatments on protein expression of cumulus cells were not found in the literature. A study on gene expression in cumulus cells present differences between COCs matured *in vivo* with and without superovulation of donor cows (Barros et al., 2012). So aberrations on protein level are likely. Therefore, the results will not be fully transferable on COCs matured without superovulation treatment.
The sample number for each of the four biological groups (successfully matured *in vivo*, successfully matured *in vitro*, failed to mature *in vivo*, failed to mature *in vitro*) was limited to five. For proper label-free quantification all samples had to be analysed in on single MS run, which can include a maximum of twenty samples.

7.2 Total protein expression

A total of 2277 proteins were quantified in the 20 cumulus samples. Considering the small amount of material due to the fact that each sample originates from one unique COC, the number of identified proteins is considered very satisfactory. Other authors reported previously the identification of 1247 resp. 4395 proteins in pooled cumulus from several hundreds of bovine COCs (Memili et al., 2007; Peddinti et al., 2010). At the time we processed the samples, no descriptions of cumulus proteome on single oocyte level was available in the literature. Pooling of samples for analysis has some benefits regarding feasibility of analysis: it reduces the amount of samples that have to be analysed, which results in reduced analysis time and experimental costs. But sample pooling possesses some important drawbacks: inter-individual variations may result in dilution of low abundant proteins and outliers may be masked. The analysis of pooled samples was considered to have only a reduced applicability for biomarker discovery (Orton and Doucette, 2013).

7.3 Differences in the cumulus proteome of *in vivo* and *in vitro* matured COCs

The research question of this project was the characterization of the cumulus proteome under two different maturation conditions (*in vivo* and *in vitro*) subject to maturation outcome. Despite the small sample amount of single cumulus complexes, tremendous differences were observed between the biological groups.

More proteins were significantly upregulated after *in vivo* than *in vitro* maturation. Previous studies on bovine cumulus cells on gene expression level observed a similar upregulation for *in vivo* matured COCs (Tesfaye et al., 2009). But also contrary results with a higher gene expression after IVM than *in vivo* maturation are available in the literature (Salhab et al., 2013). It's important to remain that the protein expression isn't obligatory correlated with gene expression (Gygi et al., 1999). No data on overall protein expression in cumulus complexes are reported up to now. Also a comparison of the cumulus proteome after *in vivo* maturation and *in vitro* maturation hasn't been reported up to now.

The results of this study, comparing cumulus from COCs successfully matured *in vivo* to *in vitro* successfully matured or *in vivo* failed to mature, showed the overrepresentation of proteins involved in the following biological pathways:

- Complement and coagulation cascades (successfully matured *in vivo* > successfully matured *in vitro* & *in vivo* failed to mature)
- Steroid biosynthesis (successfully matured *in vivo* > successfully matured *in vitro*) / Ovarian steroidogenesis (successfully matured *in vivo* > *in vivo* failed to mature)
- N-Glycan biosynthesis (successfully matured *in vivo* > successfully matured *in vitro*)
- ECM-receptor interaction (successfully matured *in vivo* > successfully matured *in vitro* & *in vivo* failed to mature)

For the *in vitro* matured group and the cumulus that failed to mature *in vivo* no enriched pathways were detected.

For these enriched pathways, similar but also contradictory results were found in human and bovine cumulus transcriptomics studies and equine proteomics study:
In human cumulus of *in vivo* matured MII oocytes, genes linked with steroid metabolism were overrepresented compared to cumulus of *in vitro* matured MII oocytes (Ouandaogo et al., 2012), which is an analogical result to this study. The opposite was observed in bovine

cumulus complexes, also on gene expression level: candidates associated with ECM-formation, steroid biosynthesis or complement and coagulation cascade were reported as upregulated after *in vitro* maturation in cattle compared to *in vivo* maturation (Salhab et al., 2013). The same methodology of this study was used to analyse the proteome of equine *in vivo* and *in vitro* matured cumulus. The results corroborate the presented bovine results with a massive overexpression of the complement and coagulation pathway in *in vivo* matured cumulus compared to *in vitro* matured (Huwiler et al., 2016).

These overrepresented pathways are mostly related to non-reproductive topics, but a relation to intrafollicular events is also documented in the literature. In the following chapters the potential role of these pathways for COC maturation and their influence on the developmental competence of the oocyte will be discussed.

7.3.1 Complement and coagulation cascades

Enrichment analysis revealed that the KEGG pathway "complement and coagulation cascades" was significantly overrepresented in *in vivo* successfully matured cumulus compared to *in vitro* successfully matured cumulus (21 proteins, $p=0.0001$, see Table 4) and *in vivo* failed to mature cumulus (21 proteins, $p<0.0001$, see Table 8). The 21 proteins are identical but fold changes for the two group comparisons were different (see Tables 4 & 8). Eight of these proteins belong to the complement system and 13 are involved in the coagulation cascades.

Complement system

The overexpressed proteins of the complement system in the *in vivo* successfully matured cumulus are: C1QC protein (C1QC), complement C3 (C3), complement C4A (C4A), C4b-binding protein alpha chain (C4BPA), complement component C7 (C7), complement component C8 alpha chain (C8A), complement component C9 (C9) and complement factor H (CFH) -with significant overexpression in comparison to cumulus of *in vitro* successfully matured oocytes as well as cumulus of oocytes that failed to mature *in vivo*. Complement system comprises over thirty proteins, participates to the innate immune system and is involved in the ovulation process (Perricone et al., 1990; Shimada et al., 2006). The overregulated proteins are involved in all the three activation pathways of the complement cascade: the classical, the alternative and the lectin pathways. All these pathways activate the

central C3 component of the complement to activate the inflammatory process (Jarkovska et al., 2010).

A certain local sterile inflammatory process occurs physiologically in the follicle around ovulation time (Boots and Jungheim, 2015; Espey, 1994; Spanel-Borowski, 2011). During maturation progress, the high metabolism generates an increased oxidative stress and danger signals are send to the innate immune system, responsible for induction of the first, inflammatory, phase of the ovulatory process (Spanel-Borowski, 2011).
Inflammatory mediators, released in response to LH-peak, induce angiogenesis, hyperemia and an enzymatic cascade that modify the follicle wall to cause ovulation (Boots and Jungheim, 2015). Members of the complement cascade are involved in follicular wall modulation and were reported in previous studies interrogating follicular fluid composition (Ambekar et al., 2013; Jarkovska et al., 2010; Twigt et al., 2012). A favouring role of the complement cascade proteins in the preovulatory environment of the oocyte on its further development potential was already reported (Gonzales et al., 1992; Hashemitabar et al., 2014; Jarkovska et al., 2010).
Proteins of the complement system are widely expressed in the female reproductive tract and were already detected in cumulus cells and in the oocyte (Georgiou et al., 2011; Shimada et al., 2006; Taylor and Johnson, 1996).
The central protein complement C3, upregulated in the present study in *in vivo* successfully matured cumulus, seems to be involved also in the fertilization process (see Chapter 7.4.7).
Expression of genes in cumulus during ovulation, which are related to innate immunity, like complement factor C1q, shows that cumulus plays more than the protective physical function for the oocyte (Shimada et al., 2006). Cumulus cells are also involved in recognition and clearance of apoptotic cells during this period (Shimada et al., 2006).
A positive influence of complement on *in vitro* maturation success was reported with a higher oocyte maturation rate in presence of non-treated follicular fluid than heat-treated one. Georgiou and coauthors explain these findings by the heat instability of complement proteins. Lower rates in the heat-treated group could be compensated by addition of iC3b, a cleavage product of C3 (Georgiou et al., 2011). Environment surrounding more competent oocytes in different studies was reported as enriched in C3 proteins (Gonzales et al., 1992; Hashemitabar et al., 2014). According to Georgiou and coworkers results, the lower C3 protein and other complement proteins concentration in cumulus from COCs that failed to mature *in vivo* in the present work may suggest the suboptimal complement expression as a possible cause for the

poor maturation outcome. Yoo and coworkers identified complement factors in human follicular fluid using LC/MS/MS protein analysis. RNA analysis of granulosa cells revealed that the complement factors are actively produced by these somatic cells (Yoo et al., 2013).
The positive correlation between upregulated complement proteins in cumulus cells and the maturational competence of the corresponding oocyte is in accordance with the available literature. It can be hypothesized, that the cumulus cells support oocyte maturation by secretion of complement factors.

Coagulation system

The proteins alpha-2-macroglobulin (A2M), prothrombin (F2), coagulation factor X (F10), fibrinogen alpha chain (FGA), fibrinogen beta chain (FGB), fibrinogen gamma-B chain (FGG), kininogen-1 (KNG1), tissue-type plasminogen activator (PLAT), plasminogen (PLG), alpha-1-antiproteinase (SERPINA1), plasma serine protease inhibitor (SERPINA5), antithrombin-III (SERPINC1), SERPIND1 protein (SERPIND1) belong to the coagulation cascades. These proteins were overexpressed in *in vivo* matured cumulus compared to *in vitro* matured or *in vivo* failed to mature cumulus. Proteins of the coagulation system were already reported to be present in bovine follicular fluid (Yamada and Gentry, 1995). A nutritional influence on coagulation protein concentration in follicular fluid has previously been reported for the porcine species (Jarrett et al., 2015). This influence can be ruled out for this study, where all heifers were housed and fed together for months prior to slaughtering. An analysis of literature regarding human follicular fluid composition reports correlations between presence of proteins from the coagulation system in follicular fluid and oocyte quality (Bianchi et al., 2016). The impact of proteins from the coagulation system in follicular fluid on positive IVF outcome in the human species was already described (Severino et al., 2013). Role of the coagulation system on ovulation process and then on oocyte delivery in the oviduct via influencing follicular fluid concistency and inflammatory cells modulation is described (Severino et al., 2013; Shen et al., 2017) but literature about the influence of proteins from the coagulation system on oocyte maturation is rare. Two proteins will be described later in this work: Antithrombin III (SERPINC1) regarding the influence on fertility such as oxidative stress defence, influence on apoptosis process and influence on sperm (see Chapters 7.4.1, 7.4.2 and 7.4.7). α2-macroglobulin (A2M), another protein of the coagulation system will be also being discussed with regard to the post ovulatory processes (see Chapter 7.4.6).

7.3.2 Steroid biosynthesis / Ovarian steroidogenesis

Steroid biosynthesis

In *in vivo* matured compared to *in vitro* matured cumulus, an overexpression of proteins active in steroid biosynthesis pathway was observed (Table 5). These overexpressed proteins are all active in cholesterol biosynthesis: squalene synthase (FDFT1) (Do et al., 2009), sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating (NSDHL) (Caldas and Herman, 2003), Lanosterol 14-alpha demethylase (CYP51A1) (Trzaskos et al., 1995), 7-dehydrocholesterol reductase (DHCR7) (Brown et al., 2014), lanosterol synthase (LSS) (Krisans, 1996), squalene epoxidase (SQLE) (Sakakibara and Ono, 1994), DHCR24 protein (DHCR24) (Brown et al., 2014). Cholesterol plays different roles like being a component of membranes or a precursor in steroid hormone production. Beside this, further functions like protection against oxidative stress and apoptosis were reported for DHCR24. For details see review by Zerenturk and coworkers (Zerenturk et al., 2013).

Similar results were found on gene expression level: Genes involved in cholesterol biosynthesis were downregulated in human cumulus after IVM compared to *in vivo* matured (Ouandaogo et al., 2012).

The underexpression of these proteins under *in vitro* maturation conditions indicates that the COC matured under non-physiological conditions may present a reduced cell-membrane quality, defects in steroidogenesis, possess reduced defence mechanisms against oxidative damages and cell death and may suffer due to such a reduced local cholesterol synthesis.

Ovarian steridogenesis

During the periovulation ovarian steroidogenesis, induced by the LH surge, is highly active (Boots and Jungheim, 2015), which requires a higher availability of the precursor cholesterol. The impact of cholesterol metabolic pathways on fertility was reviewed by De Angelis and coworkers (DeAngelis et al., 2014).

Several proteins involved in steroidogenesis are overexpressed in the cumulus of successfully *in vivo* matured oocytes of the present study, compared to cumulus of oocytes that failed to mature *in vivo*: cholesterol side-chain cleavage enzyme, mitochondrial (CYP11A1), aromatase (CYP19A1), low-density lipoprotein receptor (LDLR), prostaglandin G/H synthase 2 (PTGS2) and scavenger receptor class B member 1 (SCARB1) (Table 10). Only three of these proteins (CYP19A1, PTGS2 and SCARB1) were also significantly overexpressed in *in*

vivo matured cumulus compared to *in vitro* matured but the KEGG pathway was not considered as enriched.

To enter the steroidogenic pathways, cholesterol has to be transported to the mitochondria, where it will be converted to pregenolone that can be converted in progesterone or in DHEA and sex steroids (Conley and Bird, 1997; Dumesic et al., 2015).

The enzyme SCARB1 plays a role in cholesterol transport from peripheral tissues (Krieger, 2001). A lack of this protein was reported to impair lipoprotein metabolism, causing abnormal oocytes resulting in female infertility (Miettinen et al., 2001). The LDLR receptor is on the cell membrane and permits to transport cholesterol into the cell (DeAngelis et al., 2014). LDLR plays a role in female fertility: knock-out mice are fertile, but produce smaller litters (Guo et al., 2015). The CYP11A1 is a cholesterol cleavage enzyme that catalyses the first step of cholesterol conversion toward pregnenolone (Strushkevich et al., 2011). The enzyme aromatase (CYP19A1) catalyses the transformation of androgens into estradiol (Ghosh et al., 2009). This mechanism was attributed to granulosa cells (Hillier et al., 1994) but a local aromatase activity in cumulus cells was also described (Dumesic et al., 2015; Laufer et al., 1984). Gene expression of CYP19A1 is described as reduced in cumulus from subfertile patients, that may possibly suffer from impaired oocyte maturity (Hosseini et al., 2016).

PTGS2, also overexpressed in cumulus of successfully *in vivo* matured oocytes, catalyses the conversion of arachidonic acid into prostaglandin H2 (Williams et al., 1999). A lack of PTGS2 causes female infertility in mouse, due to implication of the protein in several steps of the reproductive process, beginning from abnormal oocytes, as reviewed by Williams and coworkers (Williams et al., 1999).

The overexpression of these proteins in the present study reveals that the ovarian steroidogenesis pathway is particularly active during *in vivo* maturation.

Variations in estradiol and progesterone synthesis were observed during the maturation process (Espey, 1994). The follicular oestrogen/progesterone ratio (E2/P4) can also be used to differentiate follicles from a same cow with different activity status (Renaville et al., 2010). These variations contribute to oocyte maturation and acquisition of developmental competence (Mingoti et al., 2002).

Previous literature describes the expression of several genes in bovine cumulus cells encoding enzymes active in steroidogenesis (Burmester-Kintrup, 2014; Salhab et al., 2011). Cumulus cells are able to secrete oestrogen and progesterone during IVM (Mingoti et al., 2002; Schoenfelder et al., 2003) even when the concentrations remain below the ones under *in vivo* conditions (Schoenfelder et al., 2003). Regarding the present results, the reduced secretion of

hormones during IVM may be related to a reduced steroidogenic machinery, as proteins involved in steroidogenesis present also a reduced expression *in vitro* compared to *in vivo*.
After IVM under different gas and oil conditions different gene expression in bovine cumulus cells regarding enzymes involved in steroidogenesis could be observed (Burmester-Kintrup, 2014). Hence, external factors, known to be detrimental to oocyte competence like heat stress, also influence expression of genes involved in steroid synthesis like CYP11A1 and CYP19A1 in follicular somatic cells (Li et al., 2016).
Local steroids don't mediate the action of gonadotropins on meiotic activation in mammals, as it is the case in fish and amphibians (Nagahama et al., 1995; Schuetz, 1974; Tsafriri et al., 2005). Even if meiosis seems to resume and complete independently of these follicular steroids, they are necessary around meiotic resumption to allow normal further development of the oocyte (Bar-Ami et al., 1983; Moor and Trounson, 1977; Tsafriri et al., 2005). Further steps from fertilization (Moor et al., 1980) up to preimplantatory embryogenesis (Borman et al., 2003) are impacted by a lack of these hormones. Steroids like progesterone produced by cumulus cells act also as chemoattractant for sperm in some mammals (Guidobaldi et al., 2008; Oren-Benaroya et al., 2008; Teves et al., 2009).

7.3.3 N-Glycan biosynthesis

The presented results revealed the overexpression of seven proteins from the N-Glycan biosynthesis pathway in the *in vivo* matured cumulus compared to cumulus from *in vitro* matured COCs: dolichyl-diphosphooligo-saccharide-protein glycosyltransferase subunit DAD1 (DAD1), dolichyl-diphosphooligo-saccharide-protein glycosyltransferase 48kDa subunit (DDOST), dolichyl-diphosphooligo-saccharide-protein glycosyltransferase subunit 1 (LOC539818), alpha-mannosidase 2 (MAN2A1), RPN1 protein (RPN1), dolichyl-diphosphooligo-saccharide-protein glycosyltransferase subunit 2 (RPN2), and STT3B protein (STT3B) (Table 6).
These proteins are all involved in the biosynthesis of Glycans. These are defined as carbohydrates bound as glycoconjugate to proteins (glycoprotein) or lipids (glycolipids) (Lee et al., 2015). Glycans attached to proteins at asparagine residues by N-glycosidic bonds are called N-linked-glycans (Apweiler et al., 1999) (Imperiali and Hendrickson, 1995; Lee et al., 2015).
Glycosylation is one of the most common forms of posttranslational modifications, where carbohydrates are enzymatically added to the proteins (Wopereis et al., 2006). The N-linked

glycosylation influences the proprieties and functions of a protein (Lee et al., 2015). At the surface of the cells, glycans are involved in cellular interactions (Moremen et al., 2012). Numerous functions in protein secretion, inflammatory process, and hormone action were reviewed in the literature (Moremen et al., 2012; Nagae and Yamaguchi, 2012).

Regarding the female reproduction, glycosylation plays a central role in the fertilization process. Gamete binding occurs on carbohydrates recognition, also in the bovine species (Defaus et al., 2016; Velasquez et al., 2007). N-glycosylation sites on bovine zona pellucida glycoproteins play a role in sperm-oocyte interactions (Yonezawa, 2014). Changes in zona pellucida glycoproteins during maturation is necessary for normal fertilization in the pig (Topfer-Petersen et al., 2008). Differences in the glycan profile of cumulus cells between species as well as between maturation conditions are described (Accogli et al., 2014). Contribution of cumulus to zona pellucida glycosylation, via synthesis and secretion of glycoproteins, influences indirectly gamete-binding processes (Accogli et al., 2014), as glycosylation of zona pellucida glycoproteins was reported as central in sperm penetration, binding and acrosome reaction (Lay et al., 2013).

An example for the importance of glycosylation processes within the cumulus oophorus is the protein glycodelin (Yeung et al., 2006). This protein is present in different glycosylation isoforms: glycodelin-A, -S, -F and C (Seppala et al., 2007; Seppala et al., 2009). The last one (glycodelin-C) is produced in cumulus cells through conversion of glycodelin-A and glycodelin-F, which are available in COC environment (Seppala et al., 2007). While traversing the cumulus mass towards the oocyte, the inhibitive glycodelin-A and glycodelin-F, that prevented premature acrosome reaction of the sperm cell, are displaced (Seppala et al., 2007) and glycodelin-C provides a stimulatory effect for the gamete binding process (Chiu et al., 2007).

N-glycosylation of some extracellular matrix proteins is also described as modulating factor of the binding between fibronectins and integrins (Nagae and Yamaguchi, 2012).

Fertilization success is related to different glycan pattern, which was described in equine and porcine as different between both species and between *in vivo* and *in vitro* maturation of the COCs. Accogli and coauthors suggest that these differences may explain the reduced potential of these species to develop successfully after *in vitro* processing (Accogli et al., 2014).

The different expression of proteins involved in the N-glycan biosynthesis in the present study indicates that maturation conditions influence the posttranslational modifications and modulate the expression and functions of the proteins. Regarding the role of glycosylation in cumulus complexes, a higher expression of proteins involved in N-Glycan biosynthesis in

cumulus from *in vivo* matured oocytes may be an advantage for sperm passage through the cumulus and binding and penetration of the oocyte compared to *in vitro* matured COCs.

7.3.4 ECM-receptor interaction

In the present study, proteins from the collagen family (collagen alpha-1 (I) chain (COL1A1), collagen alpha-1 (IV) chain (COL4A1), collagen alpha-2 (IV) (COL4A2), collagen alpha-2 (VI) chain (COL6A2), collagen alpha-3 (VI) chain (COL6A3)), integrin beta-5 (ITGB5), proteins of the laminin family (laminin subunit alpha-1 (LAMA1), laminin subunit beta-2 (LAMB2), laminin subunit gamma-1 (LAMC1)), embryo-specific fibronectin 1 transcript variant (FN1) and vitronectin (VTN) are overexpressed in *in vivo* matured cumulus compared to *in vitro* (Table 7).
In *in vivo* matured cumulus, ten (COL1A1, COL4A1, COL4A2, COL6A3, ITGB5, LAMA1, LAMB2, LAMC1, FN1, VTN) of these proteins and another one, thrombospondin-2 (THBS2) were also overexpressed compared to cumulus that failed to mature *in vivo* (Table 9).
All these proteins are involved in extracellular matrix-receptor interactions. During maturation, with its expansion of the cumulus extracellular matrix, these proteins are not only expressed to build up ECM, even more they are involved in the interaction of the matrix with the surrounding environment. Matrix molecules are described to interact with cell membrane bound receptors like integrins, which is also described for other organs and situations like cancer and inflammation (Heino and Kapyla, 2009).

Collagens, laminins, fibronectin and vitronectin are all ligands for the transmembrane receptor integrin, also overexpressed in these samples. The upregulation of expression of these proteins in the present *in vivo* successfully matured COCs correlates with previous studies.
Collagens, laminins and integrin were reported with increased expression during cumulus mucification in the cow (Sutovsky et al., 1995) as well as vitronectin and fibronectin that were also reported in bovine cumulus cells and in the cumulus ECM matrix along maturation, with an increased expression post maturation (Thys et al., 2012; Thys et al., 2009). Thrombospondin production in bovine granulosa cells was described already years ago (Bond, 1997). Another form of thrombospondin, the thrombospondin 1 was described to be overexpressed 6 hours post LH surge compared to prior LH surge (Assidi et al., 2010). In the present work, the thrombospondin 2 was overexpressed in *in vivo* matured cumulus compared

to *in vivo* failed to mature. In a study observing abnormal collagen fibrils in THBS2 deficient animals, this protein is suggested as providing information for ECM assembly (Lawler, 2000).

ECM-receptor interaction proteins play a role in post-maturation events: they are possibly involved in the maintenance of the expanded matrix around cumulus cells and oocyte, and only expanded ECM around cumulus cells permit correct adhesion and oviductal pick-up of the COC (Familiari et al., 1996; Lam et al., 2000; Relucenti et al., 2005; Talbot et al., 2003). They also influence sperm motility and are involved in gamete adhesion and fertilization process (Familiari et al., 1996; Fusi et al., 1996; Hoshi et al., 1994; Relucenti et al., 2005; Talbot et al., 2003).

Increased vitronectin in extracellular matrix is reported with negative effects on sperm motility, egg-sperm interaction (Tanghe et al., 2004). A dose dependent effect is described for vitronectin on sperm penetration, with a beneficial effect for low doses and a negative effect for higher concentrations in medium (Thys et al., 2012). Cumulus extracellular matrix plays a moderating role to capture the surplus of vitronectin (Thys et al., 2012). Cumulus enclosed and denuded oocytes are differently impacted by the fertilization medium composition. In the presence of high vitronectin concentrations, the penetration and fertilization capability of denuded oocytes are massivly reduced, but cumulus enclosed oocytes retain a certain capability compared to denuded (Thys et al., 2012). Beside the reduced fertilization and sperm penetration rate, a high reduction of polyspermy in cumulus enclosed oocytes could be observed while vitronectin concentrations was high in medium (Tanghe et al., 2004) compared to lower concentrations. In denuded oocytes, polyspermy rate remains similar under both concentrations, but fertilization and sperm penetration rate were also highly reduced compared to intact COCs (Thys et al., 2012). The inhibition rate of penetration of sperm under high vitronectin concentration was about 90% in denuded oocytes versus about 55% in COCs (Thys et al., 2012). The increased expression of an ECM-protein like vitronectin in *in vivo* matured cumulus samples may possibly be involved in reducing/avoiding the polyspermy phenomenon in bovine *in vivo* matured COCs. Under *in vitro* maturation conditions the risk for increased polyspermy rates was described in cattle (Gordon, 2003a; Hosoe et al., 2014; Leibfried-Rutledge et al., 1987; Parrish, 2014). This implication may be explained by a lack of proteins involved in ECM-receptor interaction in successfully *in vitro* matured COCs compared to successfully *in vivo* matured COCs.

7.4 Discussion of enriched single proteins

Beneath the enriched pathways, several individual proteins were significantly different expressed. Some of these proteins were chosen for discussion, as they may possess biological functions of special interest for the maturation process. A manual grouping of these individual proteins was conducted according to the potential role in the cumulus oophorus during maturation and in the post-maturation functions (Table 11).

7.4.1 Proteins involved in oxidative stress defence

Different proteins involved in oxidative stress were significantly different expressed in this study: Cystatin B (CSTB), Thiredoxin reductase 2 (TXNRD2), Copper-transport protein (ATOX1), Peroxiredoxin-6 (PRDX6), Versican core protein (VCAN), Caveolin-1 (CAV1), Antithrombin III (SERPINC1), Serotransferrin (TF), copper-zinc superoxide dismutase (SOD1). The expression of these proteins differs between the different groups (Figure 21).
Gene expression for all these proteins was already reported for bovine intrafollicular somatic cells (Khan et al., 2016). The potential biological functions for these proteins involved in oxidative stress defence in the cumulus oophorus will be described in the following paragraphs.

Cystatin B (CSTB)

Cystatin B was upregulated in cumulus from *in vitro* successfully matured COCs compared to *in vitro* failed to mature.
Cystatin-B is involved with superoxide dismutase 1 together in oxidative stress defence (Ulbrich et al., 2014). A lack of cystatin results in an increased production of free radicals like superoxide (Maher et al., 2014) and a sensitisation of cells to oxidative stress (Lehtinen et al., 2009). In cumulus cells, a reduced cystatin B expression was described in COCs from aged women (McReynolds et al., 2012). This result correlates with the present observation that cumulus surrounding less competent oocytes (*in vitro* successfully matured) was associated with lower cystatin B expression than cumulus from *in vivo* matured COCs.

Thioredoxin reductase 2 (TXNRD2)

Thioredoxin reductase 2 was upregulated in cumulus from *in vitro* successfully matured COCs compared to *in vitro* failed to mature.

The thioredoxin reductase 2 enzyme (Trx-2) belongs to the mitochondrial thioredoxin-dependent peroxide reductase system (Watabe et al., 1999). The protein family of thioredoxin reductases maintains the redox protein thioredoxin (Trx) in a reduced state via NADPH-dependent reduction (Mustacich and Powis, 2000). Different forms of the protein are described. The second one (Trx-2) is mitochondria specific (Spyrou et al., 1997; Tanaka et al., 2002) and participates in the defence against ROS-induced damages and regulates apoptotic pathway (Nonn et al., 2003; Tanaka et al., 2002). Enzymes of this system inhibit apoptosis (Baker et al., 1997; Zhang et al., 1997). Thioredoxin reductase 2 acts indirectly against oxidative stress as it maintains thiredoxin in a reduced state, or it recycles non-enzymatic antioxidants like ascorbate (Li et al., 2001).

This protective effect against oxidative stress is particularly observed in tissues with high metabolic activity or free radical exposition (Mustacich and Powis, 2000; Nonn et al., 2003; Schallreuter and Wood, 2001; Stanley et al., 2011; Watabe et al., 1999). Thioredoxin promotes cell survival of cancer cells undergoing hypoxic conditions (Hedley et al., 2004) and inhibits their apoptosis (Powis et al., 2000).

The thioredoxin system is also of importance in cumulus cells. Thioredoxin gene expression increases in bovine cumulus after maturation under favourable IVM conditions (Deb et al., 2012a). Another protein involved in the system and described in cumulus cells is the thioredoxin interacting protein (TXNIP) (Salhab et al., 2013). This protein inhibits the, in the present study upgulated, thioredoxin reductase (Salhab et al., 2013). This inhibitory protein was increased in cumulus after IVM compared to *in vivo* maturation and is suspected to have a detrimental effect on oocyte quality (Salhab et al., 2013).

Lack of Trx-2 induces increased ROS generation, supports apoptotic pathways and lead to premature death during embryonic development (Nonn et al., 2003; Tanaka et al., 2002). Retrospective examinations of embryos revealed a downregulation of the thioredoxin gene associable with a reduced development potential (El-Sayed et al., 2006). To improve embryo culture in livestock, thioredoxin was added to embryo culture medium, with positive effect on development rates (Bing et al., 2003). Overall cell number of blastocysts was improved in parallel to a reduced number of apoptotic cells (Ozawa et al., 2006).

According to previous studies (Tamura et al., 2008; Zhang et al., 2006), the present reduced TrxR-2 expression in cumulus from COCs that failed to mature *in vitro* contribute to the hypothesis that a reduced competence in the defence against oxidative stress may contribute to a failure to acquire maturational competence under *in vitro* conditions.

Copper-transport protein ATOX1 (ATOX1)

The copper-transport protein ATOX1 is overexpressed in the groups with expected lower development potential like in *in vivo* failed to mature compared to *in vivo* successfully matured as well as in *in vitro* successfully matured compared to *in vivo* successfully matured.

The antioxidant copper-transport protein ATOX1 (Hatori and Lutsenko, 2013) is limiting the oxidative damages due to a lack of SOD (Kelner et al., 2000). This protein was shown to protect SOD1 deficient yeast against superoxide anions and hydrogen peroxide induced injuries (Lin and Culotta, 1995). An increased ATOX1 concentration after *in vitro* maturation may possibly find its origin in an excess of oxidative stress under *in vitro* maturation conditions compared to *in vivo*. In mice oocytes, an increased ATOX1 expression was described in the expected less competent ones (O'Shea et al., 2012). This is in accordance with the results of this study.

Peroxiredoxin-6 (PRDX6)

Peroxiredoxin-6 was found in a significant higher amount after successful maturation *in vitro* than after *in vivo* maturation.

The protective effect of peroxiredoxin 6 on different cell types undergoing oxidative stress is well described (Asuni et al., 2015; Li et al., 2015; Singh et al., 2016; Tu et al., 2016).

In the male, a low peroxiredoxin level in gametes is associated with reduced fertility due to poor motility, increased damage on genetic material (Gong et al., 2012) and damages through oxidative stress (Ozkosem et al., 2016). Like in female (Agarwal et al., 2005), an increased oxidative stress in reproductive tract as well as a decline of gamete competence are to observe with donor age also in the male (Ozkosem et al., 2015). PRDX6 shows also a protective effect for the male gamete: in deficient males, the decline of gamete competence is amplified (Ozkosem et al., 2015). PRDX6 deficiency was described for male infertility cases (Liu and O'Flaherty, 2016) and PRDX6 levels are increased when sperm maturation occurs under oxidative stress (Liu and O'Flaherty, 2016).

The PRDX6 gene and protein expression in bovine cumulus and oocytes were described as upregulated after IVM in comparison to before maturation (Leyens et al., 2004). Increased expression in the oocytes depends on patent gap junctions, what suggest an impact from cumulus on oocyte levels (Leyens et al., 2004). Regulation of the expression in cumulus cells depends on the presence of oocytes, even when no intercellular connexion is present (Leyens et al., 2004). Peroxiredoxin-6 expression and oxidative stress are in relation: peroxiredoxin-6 overexpression is associated with resistance to oxidative stress and decreased expression to

sensitivity (Fisher, 2011). The role of peroxiredoxin-6 as antioxidant is well described (Phelan, 1999; Sparling and Phelan, 2003) and a review discusses the cytoprotective role of different enzymes of the peroxiredoxins family (Rhee, 2016). Regarding the functions in antioxidant defence, intercellular signalling and prostaglandin production (Leyens et al., 2004), an hypothesis may be that oocytes during IVM stimulate, via paracrine factors, PRDX6 expression in cumulus in order to compensate suboptimal maturation conditions.

Versican core protein (VCAN)

Versican core protein was upregulated after successful *in vivo* maturation compared to *in vitro* maturation or failure to reach MII under *in vivo* conditions.

Versican expression is described as linked with metabolically active tissues, in the skin and several tumors and is related to cell survival and protection against oxidative stress (Wu et al., 2005a). Versican gene expression is also reported as linked with a low oxygen tension (Sotoodehnejadnematalahi et al., 2015), like the preovulatory intrafollicular environment, that is described as hypoxic (Fang Yang, 2016), opposite to the higher oxygen pressure during the *in vitro* culture conditions.

Opposite to the present results, a meta-analysis reported a negative correlation between oocyte maturity and *VCAN* gene expression in human cumulus (Pourret et al., 2016). In the same analysis *VCAN* in human cumulus is also described as marker for successful further development like live birth (Pourret et al., 2016). Regarding maturation conditions and according to the present results, expression of Vcan was examined post IVM in murine cumulus and presented a >10-fold reduction compared to *in vivo* maturation (Dunning et al., 2007). The same authors described the presence of *VCAN* in *in vivo* matured human cumulus and hypothesized a role of Vcan in expansion and maturation of the COC, with a reduced Vcan expression after IVM suggesting an impaired maturation environment (Dunning et al., 2007).

Other research groups working on human presented similar results regarding the correlation between *VCAN* expression in cumulus and successful maturation (Adriaenssens et al., 2010) and were even able to predict the pregnancy outcome (Gebhardt et al., 2011). The VCAN protein expression in cumulus was also lower in COCs from donors of advanced maternal age, where a reduced developmental competence is expected (McReynolds et al., 2011). Even for the bovine species, *Vcan* was described as marker in cumulus cells for the competence of the oocyte to develop into a blastocyst (Kussano et al., 2015). The upregulation of VCAN in successfully matured COCs post *in vivo* maturation compared to post *in vitro* maturation, as

well as to COCs that failed to mature *in vivo* strengthens the presumption that *Vcan* expression is a good marker for oocyte quality and competence in various species. Two main hypotheses for the mode of action for VCAN in oocyte competence acquisition were discussed. One may be the effect as antioxidant to protect oocyte and cumulus from free radicals (Dunning et al., 2007; Wu et al., 2005a). The other could be a stabilization function of the hyaluronan in expanded cumulus extracellular matrix, which will be discussed in a later chapter of this work (Gebhardt et al., 2011; Wu et al., 2005b) (See Chapter 7.4.5).

Caveolin-1 (CAV1)

Caveolin-1 was upregulated after successful *in vivo* maturation compared to successful *in vitro* maturation and also to the group that failed to reach MII under *in vivo* conditions.

The expression of the caveolin-1 gene was already examined in bovine cumulus, as not detectable in immature COCs and with an increased expression in COCs during IVM (Rispoli et al., 2013). Higher values were measured in matured COCs with good developmental competence compared to COC challenged by heat stress (Payton et al., 2009). In bovine granulosa cells an impressive increase in caveolin-1 gene and protein expression was observed towards ovulation, with a 6.5 fold higher gene expression in ovulatory follicles than in dominant follicles (Diouf et al., 2006).

Caveolins are scaffolding proteins that are independent of caveolae or components of caveolae in plasma membrane (Liu et al., 2002; Zheng et al., 2011). These proteins are involved in signal transduction (Cohen et al., 2004; Liu et al., 2002; Razani et al., 2002b; Williams and Lisanti, 2005), vesicular transports like endocytosis (Pelkmans et al., 2004; Quest et al., 2004) and cholesterol trafficking (Bosch et al., 2011; Liu et al., 2002; Schlegel et al., 2000). Caveolin-1 seems to act on prostaglandin production around ovulation (Diouf et al., 2006). There seems to be an interaction with PTGS2 (Liou et al., 2001). Both proteins present also parallel variations in expression between the different groups in the present study.

Depending on the tissue, caveolin-1 can act pro- or anti-apoptotic, with modulating effects on cell proliferation through actions on growth factors (Williams and Lisanti, 2005). Variations in growth factor signalling around ovulation time may be the way in which caveolin-1 modulates the COCs development potential (Diouf et al., 2006). Caveolin-1 deficiency was linked to misbalances in lipid storage and utilisation (Bosch et al., 2011; Pavlides et al., 2010; Razani et al., 2002a), increased oxidative stress similar to hypoxia, abnormal nitric oxide content (Garcia-Cardena et al., 1997; Pavlides et al., 2010; Razani et al., 2001; Zhao et al., 2009) and abnormal calcium signalling (Liu et al., 2002). Oxidative stress induces a reduced

expression of caveolin-1 (Mougeolle et al., 2015). The same authors hypothesized that a caveolae-dependent pathway may be involved in regulation of oxidative stress in muscle cells. A downregulation of caveolin-1 expression was linked to autophagy (Shi et al., 2015), a lysosomal pathway into cytoplasm for elimination of damaged content and maintenance of cytoplasmic homeostasis (Rubinsztein et al., 2012). Through autophagy, nutrients are recycled as survival strategy under difficult conditions (Han et al., 2011; Loos et al., 2013; Shi et al., 2015). Autophagy was also described for granulosa cells and for the elimination of oocytes in follicular atresia (Escobar et al., 2013). These descriptions of caveolin-1 functions in different cell types correlate with the increased caveolin-1 protein expression in cumulus from COCs with high development potential (*in vivo* matured) of this study. The lower caveolin-1 protein expression in COCs in the other groups may be associated with the expected reduced development potential of the oocytes in these groups.

Antithrombin III (SERPINC1)

Antithrombin III was upregulated after successful *in vivo* maturation compared to successful *in vitro* maturation or failure to reach MII under *in vivo* conditions.

Antithrombin III plays also a role in oxidative stress in several non-reproductive organs. Increased apoptosis and oxidative stress injuries were detected in rats with antithrombin III deficiency (*SerpinC1*) following ischemia and reperfusion (Wang et al., 2015). Negative effects of a reduced antithrombin III activity and positive effects of antithrombin III supplementation were reported in acute kidney injury (Lu et al., 2017). Complete absence of antithrombin III, as simulated in *SerpinC1* knockout rats, resulted in early embryonic loss (Wang et al., 2015). The contribution of antithrombin III to higher development competence of *in vivo* matured oocytes may also find its origin in a better response to oxidative stress. Other functions of the protein regarding sperm attraction will be described in a later chapter.

Serotransferrin (TF)

Serotransferrin was upregulated after successful *in vivo* maturation compared to successful *in vitro* maturation or failure to reach MII under *in vivo* conditions.

Serotransferrin, also called transferrin, is described as iron binding protein, responsible for iron transport. Serotransferrin stimulates cell proliferation. Gene expression of serotransferrin in different mammalian granulosa cells, including bovine, was described in the literature, with increased expression in follicles at maturation stage *in vivo* (Briggs et al., 1999; Dias et al., 2014; Nivet et al., 2013). In human serum, which reflects follicular fluid composition, more

transferrin was detected in younger patients (Hashemitabar et al., 2014). A negative effect of aging on transferrin secretion was hypothesized: a reduced expression in serum as well as in follicular fluid correlates with a lower number of successfully matured oocytes (Hashemitabar et al., 2014).

Beside the role in iron transport, a non-enzymatic antioxidant role against oxidative stress is suggested, due to conversion of oxygen peroxide to hydroxyde (Nivet et al., 2013). Transferrin is an abundant protein in tubal fluid, which contributes to the defence of the COC and embryo against oxidative stress. This is achieved via chelation of metal anions to avoid ROS production under *in vivo* conditions (Guerin et al., 2001).

Copper-zinc superoxide dismutase (SOD1)

Significant higher expression in *in vivo* successfully matured cumulus could be observed regarding the cytoplasm located copper-zinc superoxide dismutase compared to *in vitro* successfully matured.

All three isoforms of the antioxidant enzymes superoxide dismutase (SOD) were detected in the analysed cumulus samples (Zelko et al., 2002). In cumulus matured successfully *in vivo* compared to successfully matured *in vitro* the significant upregulated isoform was the copper-zinc superoxide dismutase SOD1. SOD enzymes possess a protective role during hypoxia, hyperoxia and oxidative stress-induced damages (Zaghloul et al., 2012; Zaghloul et al., 2014). A lack of SOD enzymes causes growth retardation up to death. These effects can be compensated by adding another antioxidant like ascorbic acid (Tamari et al., 2013).

All these proteins act as antioxidant enzyme that catalysis the destruction of superoxide radicals in hydrogen peroxide and oxygen (Ho et al., 1998; Zelko et al., 2002). Afterwards, H_2O_2 can be degraded via enzymes like peroxidases.

Expression of the protein seems to respond to oxygenation conditions in non-reproductive tissues (Jackson et al., 1996). Expression can be stimulated via agents responsible for cell damage like ROS (Meyrick and Magnuson, 1994). In the presence of ROS, SOD regulates gene expression toward oxidative resistance and repair mechanisms (Tsang et al., 2014).

SOD1 knockout mice have a reduced female fertility (Ho et al., 1998; Matzuk et al., 1998). Even when cycles are present and ovulation and fertilization occurs, the embryonic loss rates are higher in these mice (Ho et al., 1998). The presented results for bovine cumulus cells can lead to the hypothesis that a reduced quality of oocytes may also be involved in this reduced fertility. In the human ovary, SOD localisation rises the hypothesis for a supportive role in oocyte development (Shiotani et al., 1991).

SOD gene expression were described in previous literature in different species in immature oocytes (El Mouatassim et al., 1999; Lequarre et al., 2001), *in vivo* matured (El Mouatassim et al., 1999; Livingston et al., 2009) and *in vitro* matured oocytes (Leoni et al., 2007; Lequarre et al., 2001) with COCs from different origins (IVF after natural cycles versus hyperstimulation) (Papler et al., 2014) as well as in embryos *in vivo* and *in vitro* cultured (Lequarre et al., 2001). Regarding the *in vivo* and *in vitro* conditions, presence of SOD gene transcripts in *in vitro* as well as *in vivo* derived bovine embryos is described but the activity wasn't measured (Lequarre et al., 2001).
SOD enzymatic activity was already detected in bovine oocytes as well as in bovine cumulus matured under *in vitro* conditions (Cetica et al., 2001) but without comparison with *in vivo* counterpart.

Differences in SOD expression for different maturation conditions were already detected in other studies. *In vitro* culture conditions impaired gene and protein expression of Mn-SOD compared to *in vivo* culture (Lequarre et al., 2001). Tatemoto and coworkers described an inhibited SOD expression during maturation under *in vitro* conditions, which lead to higher superoxide radicals concentrations which resulted in disturbance of the maturation process (Tatemoto et al., 2004).
Expression of SOD seems also related to the developmental potential of the oocyte. Variation of the gene expression between individual oocytes and embryos was observed and Lequarré and coworkers suggested that SOD expression might reflect the further development potential (Lequarre et al., 2001). Addition of SOD to embryo culture media resulted in increased developmental rates of bovine (Iwata et al., 1998; Lauria et al., 1994) and murine embryos (Chun et al., 1994; Noda et al., 1991; Nonogaki et al., 1992). Other studies gave controversial results (Ali et al., 2003). Regarding maturation step, addition of Cu-Zn-SOD to IVM medium improved oocyte developmental potential in cattle (Blondin et al., 1997; Luvoni et al., 1996).
In the porcine species, oocyte's developmental competence post-maturation in SOD rich (porcine follicular fluid, pFF) and poor medium was examined (Tatemoto et al., 2004). Poor SOD conditions were related arrested meiotic progression, DNA damage in oocytes and cumulus cells, reduced further fertilization competence and general reduced development potential (Tatemoto et al., 2004).
Addition of SOD rich porcine follicular fluid during IVM protects oocyte and cumulus against oxidative stress (Tatemoto et al., 2004) with beneficial effect on maturation success (Rath et al., 1995; Vatzias and Hagen, 1999; Yoshida et al., 1992). Cumulus cells also present

a protective role against oxidative stress, which was shown for porcine oocytes undergoing oxidative stress during IVM (Tatemoto et al., 2004; Tatemoto et al., 2000). An efficient antioxidant defence system in cumulus may therefore contribute to improved oocyte developmental potential.

The increased SOD activity related to a positive outcome after ART attest to the importance of oxidative stress defence during the maturation process (Matos et al., 2009). SOD expression in cumulus decreases with female age (Matos et al., 2009), as oocyte competence decrease also in older patients. The present results correlates to the observation that more competent COCs (*in vivo* successfully matured) present a higher expression of SOD compared to less competent ones (*in vitro* successfully matured).

Oxidative stress and COC maturation: Possible implication of oxidative stress and oxidative stress defence during maturation on oocyte development competence

Different maturation conditions influence oxidative stress. *Ex vivo* culture is associated with higher oxygen concentrations, exposition to visible light, metallic cations in media, aberrant metabolism, atmospheric pollutants and reduced defence mechanisms against oxidative stress. Regarding the *in vitro* maturation condition, the results of this study suggest a reduced antioxidant activity in cumulus. This reduced defence, parallel to a probable increased ROS production due to ambient oxygen concentration, are two factors described as inducing senescence (Allen, 1998; Lu and Finkel, 2008). While ageing is associated with reduced potential (Miao et al., 2009) an increased antioxidant activity could avoid premature ageing and its deleterious effects.

Oxidative stress plays an important role during COC maturation, which may result in arrest of maturation, altered spindle morphology, DNA damage, aneuploidy, apoptotic signals and reduced oocyte developmental competence.

Cumulus cells of developmental competent oocytes seem to contribute to oocytal health by providing a better oxidative stress defence to their oocytes. An efficient antioxidant defence system in cumulus may therefore improve oocyte development potential.

7.4.2 Modulation of apoptosis

Two proteins with different influence on cell death process also differed in their expression in cumulus. The protein expression of antithrombin III is exactly opposite to the expression of Caspase-3 and this difference match with the function of these proteins in cell death process.

Antithrombin III is described as antiapoptotic and protecting cells against damages, acting via prostaglandine on cell injury and oxidative stress, and with anti-inflammatory role (Wang et al., 2015). Caspase-3 is described as the last protein activated in a cascade of different caspase proteins and is that way involved in the ultimate step of cell death process when activated (Reed, 2000).

Caspase-3 (CASP3)

In the successfully matured COCs, a significant upregulation of the caspase-3 expression in the cumulus was observed after IVM compared to *in vivo* maturation (Figure 22). The cumulus from COCs that failed to mature *in vivo* present also an increased expression compared to those that matured successfully *in vivo* (Figure 22).

Caspase-3 gene expression was detected in bovine granulosa cells (Khan et al., 2016) and cumulus cells with a decreased expression for cumulus accompanying competent oocytes (Deb et al., 2012b), which is in accordance with the presented observations.

The function of Caspase-3 effector protein in apoptotic processes is well described (Reed, 2000) and it can be considered as marker for cell death (Saraste and Pulkki, 2000). Caspases induce morphological and biochemical changes in the cell during apoptosis (Reed, 2000). An increased expression of caspase-3 in cumulus cells after IVM in presence of a toxic chemical correlates with the higher apoptosis rate in these cells (Liu et al., 2015). It was suggested that cumulus cells play a role as effect transmitter: the toxic chemical has a negative impact on oocyte maturation, which is transmitted indirectly via apoptosis in cumulus (Liu et al., 2015). Similar observation regarding the role of cumulus cells in transmission of toxic media effects to the oocyte in cattle was published earlier by Pocar and coworkers (Pocar et al., 2005).

Moreover, cumulus cells play a protective role for the oocytes against apoptosis during IVM, regarding oxidative stress defence (Tatemoto et al., 2004; Tatemoto et al., 2000): denuded porcine oocytes have an increased caspase-3 activity compared to cumulus enclosed ones (Tatemoto et al., 2000).

Apoptosis in bovine cumulus cells increases progressively during IVM (Ikeda et al., 2003). IVM COCs have an increased apoptosis rate in cumulus cells compared to immature or *in vivo* matured COCs (Salhab et al., 2013). As *in vivo* maturation is not expected to induce apoptosis in cumulus cells (Ikeda et al., 2003; Szoltys et al., 2000), the artificial maturation conditions seems to be responsible for this non-physiologic outcome (Ikeda et al., 2003). This observation corresponds to the present finding where the present apoptosis related protein was upregulated after *in vitro* maturation compared to *in vivo* maturation.

Ikeda and coworkers suggest that viability of cumulus cells undergoing IVM and developmental competence of the corresponding oocyte correlates (Ikeda et al., 2003). In *in vitro* maturation conditions that induced less apoptosis in cumulus, COCs achieved a higher developmental competence (Ikeda et al., 2003).

For bovine (Boruszewska et al., 2015; Ikeda et al., 2003; Pocar et al., 2005; Yuan et al., 2005) as well as human COCs (Host et al., 2002; Host et al., 2000; Lee et al., 2001), increased apoptosis in cumulus post maturation was related with a reduced developmental competence of the accompanying oocyte. And in the opposite, reduced apoptosis rates in cumulus were associated with a better developmental potential.

In human assisted reproduction, apoptotic markers in cumulus cells like Caspase-3 were already used for selection of oocytes with reduced developmental competence (Bosco et al., 2015; Ruvolo et al., 2015). The present results also confirm that COCs with low caspase-3 expression in the cumulus were collected from the groups with the best maturation condition and outcome (successfully *in vivo* matured) compared to the other groups (successfully *in vitro* matured of failed to mature *in vivo*).

7.4.3 APEX1, Repair of DNA damage

DNA-(apurinic or apyrimidinic site) lyase (APEX1)

In cumulus that matured successfully *in vitro*, compared to successfully *in vivo* matured, APEX1 was one of the upregulated proteins (Figure 23). APEX1 was already described in oocytes as protein for the repair of DNA damages. These damages may occur in presence of oxidative stress, which is likely present under *in vitro* maturation conditions (Bilotto et al., 2015; El-Mouatassim et al., 2007; Menezo et al., 2007). Gene expression for *Apex1* was already described in bovine granulosa cells, where older cows had a higher *Apex1* expression compared to younger cows (Khan et al., 2016).

The upregulation of APEX1 after *in vitro* maturation in this study correlates with previous results of overexpression of other genes involved DNA repair in human cumulus after IVM (Ouandaogo et al., 2012).

I*n vitro* maturation occurs under increased ROS concentration compared to *in vivo* maturation. Damages under these non-physiological conditions were described for the gametes. The enzyme encoded by *APEX1* repairs the apurinic or apyrimidinic site, typical damages caused by ROS on DNA (Hsieh et al., 2001). APEX1 and thioredoxin are both active in combination

to influence the redox potential and protect cells from programmed death (Hedley et al., 2004; Powis et al., 2000).

So the ovexpression of APEX1, which is involved in the repair of DNA damage, in the present results suggest an increased need to compensate damages after IVM than after *in vivo* maturation. Therefor it can be hypothesized that *in vitro* maturation conditions may cause more DNA damages than *in vivo* conditions.

7.4.4 Proteins involved in gas transport: Hemoglobin subunits

Hemoglobin subunit alpha (HBA) and **Hemoglobin subunit beta (HBB)**

Different components of hemoglobin were upregulated in cumulus that matured successfully *in vivo* compared to successfully *in vitro* matured and *in vivo* failed to mature (Figure 24). Both proteins were expressed with a similar pattern: Hemoglobin subunit alpha (HBA) as well as Hemoglobin subunit beta (HBB). These proteins are constituents of the hemoglobin molecule: four polypeptides subunits, two alpha and two beta subunits are necessary to build together with hem one hemoglobin molecule.

Presence of haemoglobin could be due to an impurity from preparation but few reports describes haemoglobin expression in non blood cells, also in cumulus cells (Braga et al., 2016).

Descriptions of such non-erythrocyte locations of hemoglobin genes were already described for oocyte (Labas et al., 2018) and granulosa cells in bovine (Khan et al., 2016) and cumulus cells from different mammals (Brown et al., 2015; Kind et al., 2013; Tesfaye et al., 2009). According to the present results, higher gene expression was reported in cumulus cells after *in vivo* maturation compared to *in vitro* maturation (Brown et al., 2015; Kind et al., 2013; Tesfaye et al., 2009). Hemoglobin content seems to have a beneficial effect during COC maturation under certain circumstances: addition of hemoglobin to the maturation medium increased hemoglobin in the IVM COC and, depending on the haemoglobin form used and on oxygen concentrations, may improve the developmental to blastocysts (Brown et al., 2015).

The function of this gas-binding molecule is well described for other tissues. Beneath the transport of oxygen it is involved in binding of nitric oxide and reactive oxygen species (Brown et al., 2015; Thompson et al., 2015). Therefore it acts as antioxidant and regulates apoptosis, but participates also in different metabolic pathways (Brown et al., 2015; Thompson et al., 2015).

Different hypothesis were discussed to explain the presence of hemoglobin proteins within ovarian somatic cells (Brown et al., 2015; Kind et al., 2013; Thompson et al., 2015). An action on NO is suspected, which is known as playing a role in regulation of meiosis. Binding oxygen around ovulation time may provide it for energy production (Brown et al., 2015), prevent oocyte hypoxia (Kind et al., 2013) or help to differentiate follicular cells toward corpus luteum (Brown et al., 2015). These possible roles of hemoglobin were reviewed from Thompson and coworkers, as well as the paradoxon of oxygen requirements in the avascular location of the oocyte (Thompson et al., 2015). Oxygen concentration during maturation impacts COC developmental potential (Bermejo-Alvarez et al., 2010; Hashimoto et al., 2000a; Pinyopummintr and Bavister, 1995; Watson et al., 2000). Gene expression, like those involved in metabolism, differs in bovine cumulus exposed to different oxygen concentrations during maturation (Bermejo-Alvarez et al., 2010).

The maturation conditions seems to influence the cells response to gas concentration. Cumulus cells matured *in vivo* seem to be impacted less compared to IVM cumulus which expresses hypoxia-inducible factor (HIF) under low-oxygen IVM conditions, comparable to the presumed oxygen concentration in follicular fluid around maturation time (Kind et al., 2015; Tam et al., 2010). *In vivo* and *in vitro* matured COCs present different HIF protein expression, Kind and coworkers explain this by a different response of cumulus to different environment (Kind et al., 2015).

Hemoglobin in cumulus cells seems to be responsible for the resistance against a reduced oxygen environment, ensuring the necessary oxygen supply for the oocyte and regulating the impact of oxidative stress products (Thompson et al., 2015). The expression of gas binding molecules may therefore play an important role for the further developmental potential of COCs via modulation of gas exposition for cumulus cells and oocyte. This influences as a consequence other processes like gene expression, metabolic activity and finally the maturation success.

7.4.5 Proteins involved in stability and expansion of the cumulus

Several proteins involved in stability and expansion of the cumulus complex were overexpressed in cumulus from *in vivo* successfully matured COCs compared to *in vitro* successfully matured COCs or COCs that failed to mature *in vivo* (Figure 25): inter-alpha-trypsin inhibitor heavy chain H1 to H4 (ITIH1, ITIH2, ITIH3, ITIH4), pentraxin-related

protein (PTX3), tumor necrosis factor alpha induced protein 6 (Tsg-6) and prostaglandin G/H synthase 2 (PTGS2).
The extracellular matrix surrounding the cumulus cells protects the gamete physically and guides the oocyte towards the site of fertilization after ovulation.
This matrix structure contents carbohydrates like hyaluronan and proteins like tumor necrosis factor alpha induced protein 6 (Fulop et al., 2003; Mukhopadhyay et al., 2004) or pentraxin 3 (Varani et al., 2002). These proteins are involved in matrix stabilization and are necessary to ensure the oocytes developmental competence (Ikawa et al., 2010).

Gene expression of the mentioned proteins was previously documented in bovine intrafollicular somatic cells (Khan et al., 2016).
According to the presented underexpression of these proteins after IVM, the downregulation of these genes involved in extracellular matrix composition in cumulus cells of various species after IVM was recently reviewed (Brown et al., 2017)

In the following paragraphs, the individual proteins will be discussed regarding their potential biological functions.

Tumor necrosis factor alpha induced protein 6 (Tsg-6)
Tumor necrosis factor alpha induced protein 6 was overexpressed in cumulus from *in vivo* matured COCs compared to *in vitro* matured COCs or COCs that failed to mature *in vivo*.
Tumor necrosis factor alpha induced protein 6 plays a role in cumulus ECM stability (Mukhopadhyay et al., 2004). The gene was overexpressed in the developmental more competent *in vivo* matured COCs (Tesfaye et al., 2009). A lack of TSG-6 has negative effects like a detaching cumulus after ovulation, which was linked with a reduced chance to be picked-up by oviductal fimbria. Premature oocyte denudation and a reduced fertilizability may be explanations for the sterility of knockout mice (Fulop et al., 2003). Fulop and coworkers hypothesise that a TSG-6 deficiency could also cause unexplained infertility in human (Fulop et al., 2003).
The encoding gene is considered as a marker for cumulus expansion (Hung et al., 2015). The TSG-6 proteins are only expressed after gonadotropin stimulation during the mucification process (Fulop et al., 1997; Sayasith et al., 2008). They are necessary for the formation of a stable cumulus extracellular matrix by crosslinking between hyaluronan and the heavy chains of inter-alpha-trypsin inhibitor (Carrette et al., 2001; Fulop et al., 2003; Mukhopadhyay et al.,

2001; Nagyova et al., 2008).
Even when the amount of TSG-6 from cumulus cells seems to be sufficient for mucification, granulosa cells contribute *in vivo* to an additional stabilisation of the cumulus matrix. This results in a much more resistant matrix than post IVM (Chen et al., 1996; Fulop et al., 2003).

This fact corroborate a personal observation made while sampling cumulus for this study, *in vivo* expanded cumulus presented a high sticky consistency compared to the *in vitro* counterpart. Similar observation concerning the physical proprieties regarding resistance and elasticity of COCs under both maturation conditions were already described 20 years ago (Chen et al., 1996). The different TSG-6 expression between the groups corroborates this observation and may possibly be involved in this difference between *in vivo* and *in vitro* matured COCs.

This observation is also valid for some others components of the extracellular cumulus matrix that presented a similar expression pattern, which are discussed in the following paragraphs.

Inter-alpha-trypsin inhibitor heavy chain H1 to H4 (ITIH1, ITIH2, ITIH3, ITIH4)
Inter-alpha-trypsin inhibitor heavy chain H1 to H4 were overexpressed in cumulus from *in vivo* successfully matured COCs compared to *in vitro* successfully matured COCs or COCs that failed to mature *in vivo*.
The five different members of the family: ITIH1 (Accession: sp|Q0VCM5|ITIH1_BOVIN), ITIH1 (Accession: tr|F1MMP5|F1MMP5_BOVIN), ITIH2, ITIH3 and ITIH4 presented the similar overexpression in cumulus from successfully *in vivo* matured COCs.
Beside hyaluronic acid (HA) and Tsg-6, inter-alpha-trypsin heavy chains are components of the extracellular matrix that are necessary for successful maturation.
Inter-alpha-trypsin inhibitor heavy chains proteins linked with HA were found in *in vivo* and *in vitro* expanded cumulus complexes (Chen et al., 1994; Nagyova, 2015). Expression was increased after cumulus expansion compared to compact COCs (Nagyova et al., 2004; Yi et al., 2008).
Older publications (Chen et al., 1994; Chen et al., 1996; Hess et al., 1999; Huang et al., 1993) as well as a more recent review (Nagyova, 2015) describe the functions of these proteins in mammalian COCs. These proteins are necessary for stabilization of the cumulus extracellular matrix (Chen et al., 1994; Chen et al., 1996; Nagyova, 2015) by crosslinking the HA (Chen et al., 1994; Huang et al., 1993; Nagyova, 2015). They are also responsible for the successful

expansion of cumulus (Nagyova, 2015). In absence of these proteins, HA from the matrix gets lost in the medium (Nagyova, 2015).

When IVM occurs under different conditions, cumulus matrix structure and HA accumulation within the matrix differs (Nagyova, 2015). These ITIH1-4 proteins are present in serum as well as in follicular fluid (Camaioni et al., 1993; Chen et al., 1994; Nagyova, 2015). Presence of serum or follicular fluid in media results in a more stable cumulus matrix in comparison to media that use protein substitutes as polyvinylpyrrolidone (PVP), bovine serum albumin (BSA) or polyvinyl alcohol (PVA) (Chen et al., 1992; Nagyova, 2015; Nagyova et al., 1999). When IVM occurs in the absence of serum, follicular fluid may also support the mucification process (Kimura et al., 2002; Nagyova et al., 2004). The different incorporation of inter-alpha-trypsin inhibitors in the cumulus matrix was reported to be responsible for the differences observed between physical resistance of the COCs matured *in vivo* and *in vitro* (Chen et al., 1996).

Here again, the different expression of these ITIH proteins, may explain the observation that *in vivo* expanded cumulus presented a more sticky consistency compared to the *in vitro* ones.

Pentraxin-related protein PTX3 (PTX3)

Pentraxin-related protein was overexpressed in cumulus from *in vivo* successfully matured COCs compared to *in vitro* matured COCs and COCs that failed to mature *in vivo*.

At gene level, a meta-analysis reported a correlation between oocyte maturation and *PTX3* expression in human cumulus (Pourret et al., 2016).

This protein is described in response to inflammation in different tissues. In diverse cell types PTX3 expression is induced as response to primary inflammatory mediators to protect against damages and to modulate apoptosis (Salustri et al., 2004). The analogy between inflammation and ovulation were highlighted in several publications (Espey, 1994; Richards et al., 2002).

Beside the function in inflammation, a function in fertility was also described, as cumulus cells produce PTX3 during the mucification process (Kind et al., 2013; Varani et al., 2002). The protein is localized in the extracellular matrix (Salustri et al., 2004). PTX3 plays its role in stabilisation of the matrix by building complexes with TSG6, which are responsible for HA chain cross-linking (Relucenti et al., 2005; Salustri et al., 2004). In PTX3 deficiency, hyaluronan is still produced but the organisation in a stable matrix is impaired (Salustri et al., 2004). According to the present results, gene expression of PTX3 is described as higher in murine cumulus from *in vivo* successfully matured COCs than after IVM (Kind et al., 2013). Mice with PTX3 defect are infertile due to the abnormal cumulus oophorus that prevents

fertilization *in vivo*. Still, IVF is possible for these oocytes (Salustri et al., 2004; Varani et al., 2002). Species specific differences need to be considered: in contrast to cattle, presence of cumulus isn't mandatory for IVF success in murine species (Vergara et al., 1997). Salustri and coworkers hypothesised after observation of PTX3 expression in human cumulus, that deficiencies may cause infertility due to impaired cumulus structure when fertilization happens *in vivo* (Salustri et al., 2004). A correct cumulus mass is necessary to permit an oocyte to reach fertilization location and to be fertilized successfully *in vivo* but human IVF doesn't require the presence of cumulus to be successful, as embryos are successfully produced also after cumulus removal (Van de Velde et al., 1998). Such reduced cumulus proprieties impact more the *in vivo* embryo production and *in vitro* embryo production is suggested as treatment to produce embryos from patients where infertility may be due to such a deficiency in proteins involved in cumulus structure (Salustri et al., 2004).

Pentraxin-related protein expression is impacted by maturation conditions: increased expression was described in cumulus when a medium with positive impact on oocyte developmental competence was used (Deb et al., 2012a). Suboptimal conditions may cause a decreased expression as shown in mice COCs challenged by high levels of palmitic acid, a known factor for subfertility (Wu et al., 2012a). Challenged COCs present a poor cumulus mucification and a reduced PTX3 protein expression in extracellular matrix (Wu et al., 2012a). The cumulus matrix showed PTX3 protein expression after both *in vivo* maturation and IVM, but fewer proteins are expressed in the presence of high doses of palmitic acid (Wu et al., 2012a). IVF showed an impaired developmental potential of these challenged COCs (Wu et al., 2012a). PTX3 was suggested already as marker for COC expansion (Hung et al., 2015), and development potential of the oocyte (Gebhardt et al., 2011; Zhang et al., 2005).

As already hypothesized for other proteins (Tsg-6, ITIH 1 to 4), the decreased expression of PTX3 may explain the different consistency observed during sampling of cumulus post IVM. The overexpression in *in vivo* matured cumulus may explain the higher fertility of these COCs compared to *in vitro* matured. This is in accordance to the discussion of Salustri and coworkers about the pentraxin-related protein PTX3 in cumulus from other mammals (Salustri et al., 2004).

Beside successful maturation, a possible role of pentraxin-3 may be the interaction with spermatozoa as well as the fertilization process itself (Kind et al., 2013; Salustri et al., 2004;

Varani et al., 2002).

Prostaglandin G/H synthase 2 (PTGS2)

Prostaglandin G/H synthase 2 was overexpressed in cumulus from *in vivo* successfully matured COCs compared to *in vitro* successfully matured COCs or COCs that failed to mature *in vivo*.

Prostaglandin G/H synthase 2 (PTGS2) also called COX2, is involved in prostaglandin E2 (PGE2) synthesis (Calder et al., 2001; Duffy et al., 2005; Feuerstein et al., 2007; Sirois et al., 2004). It has an impact on the cumulus mucification process (Calder et al., 2001; Davis et al., 1999; Eppig, 1981; Hizaki et al., 1999; Lim et al., 1997; Takahashi et al., 2006), maturation and oocyte quality (Gebhardt et al., 2011).

PTGS2 presents an increased expression around maturation: PGE2 in follicular fluid increases during *in vivo* maturation (Liu and Sirois, 1998; Sirois, 1994) and accumulates in *in vitro* maturation medium (Gurevich et al., 1993; Gurevich and Shemesh, 1994).

Deficiency in Cox-2 impacts fertility, acting on different early reproductive events like meiotic resumption, ovulation and fertilization processes (Lim et al., 1997). Inhibition of PTGS2 impacts negatively maturation timing (Marei et al., 2014), cumulus expansion and further developmental competence (Nuttinck et al., 2011). Lack in PTGS2 impairs cumulus expansion (Davis et al., 1999).

Addition of PGE2 to maturation media improves maturation rates (Marei et al., 2014).

PTGS2 inhibitors were used successfully in contraception in women, which is based on inhibition of ovulation and a defect cumulus oophorus (Duffy, 2015).

Several evidence of PTGS2 gene expression for the bovine cumulus exists in the literature (Brisard et al., 2012; Brisard et al., 2014; Deb et al., 2012a; Nuttinck et al., 2011; Nuttinck et al., 2008; Salhab et al., 2013). It was even considered as a marker gene for COC expansion (Hung et al., 2015). Expression in cumulus may be related to the developmental competence of the corresponding oocyte, as PTGS2 gene expression was higher in matured COCs than in immature ones (Nuttinck et al., 2008). Expression was also higher under maturation conditions that lead to an increased oocyte developmental competence (Deb et al., 2012a). According to the presented protein expression, an overexpression of the gene in *in vivo* matured bovine cumulus compared to *in vitro* matured was already reported (Salhab et al., 2013). In a study regarding a fertility-linked haplotype, a reduced PTGS2 expression on gene and protein level was observed in COCs from cows with reduced fertility compared to highly fertile cows (Brisard et al., 2014).

A similar correlation between PTGS2 expression in cumulus cells, oocyte maturity and developmental competence was observed in women (Pourret et al., 2016): the gene expression increased with progressing maturation, with highest levels in MII COCs (Feuerstein et al., 2007). PTGS2 was also described as predictor for embryo quality, successful pregnancy and live birth (Gebhardt et al., 2011; McKenzie et al., 2004). All these results correlate with the protein expression observed in the present study with a higher protein expression in the cumulus from COCs that reached successfully MII *in vivo* compared to those that failed to or those that matured under *in vitro* conditions.

Versican core protein (VCAN)

Previously described for its implication in oxidative stress defence, versican is also described as binding hyaluronan (LeBaron et al., 1992; Matsumoto et al., 2003; Sotoodehnejadnematalahi and Burke, 2013). It is necessary for hyaluronan stabilization in expanded cumulus (Gebhardt et al., 2011; Wight, 2002). Versican can also bind other proteins upregulated in the present study like fibronectin or integrin (Wu et al., 2005b). A direct influence of versican on oocyte further development potential was already suggested (Gebhardt et al., 2011).

Expression of proteins involved in stability and expansion of the cumulus oophorus is altered by maturation conditions

Different maturation conditions influence physical proprieties of cumulus, with different expansion or stability between cumulus matured *in vivo* and under different *in vitro* conditions. Chen and coworkers already reported this in the early nineties (Chen et al., 1992; Chen et al., 1990). Some of the upregulated proteins in the presented study were already suspected to be involved in these physical proprieties (Chen et al., 1992; Chen et al., 1994; Chen et al., 1996).

All the individual proteins involved in cumulus extracellular matrix formation possess a similar expression pattern in the present study, with overexpression in cumulus from *in vivo* successfully matured COCs compared to *in vitro* matured COCs or COCs that failed to mature *in vivo*. A stable extracellular matrix impacts the further post-ovulatory processes, protecting the gamete physically toward its journey to the site of fertilization after ovulation.

The overexpression of these proteins in *in vivo* successfully matured cumulus correlates with the observations made on cumulus during the sampling process for this study. This correlation suggests that maturation conditions and outcome impact stability and expansion of the

cumulus oophorus via expression of different proteins involved in stability and expansion of the cumulus.

7.4.6 Proteins involved in post-ovulatory processes

Several proteins involved in post-ovulatory processes like oviductal pick-up, journey of the COC within the oviduct and fertilization process were found as differently expressed between the groups (Figure 26): Embryo-specific fibronectin 1 transcript variant (FN1), α2-macroglobulin (A2M) and CD9 antigen (CD9) were all overexpressed in *in vivo* successfully matured cumulus compared to cumulus from COCs that failed to mature *in vivo*. FN1 and A2M were also overexpressed in *in vivo* successfully matured cumulus compared to cumulus from COCs that matured successfully *in vitro*.
All three genes encoding for these proteins were already detected in bovine intrafollicular somatic cells (Khan et al., 2016).

In the following paragraphs, the individual proteins will be discussed regarding the potential functions in post-ovulatory processes.

Embryo-specific fibronectin 1 transcript variant (FN1)
Embryo-specific fibronectin 1 transcript variant was overexpressed in *in vivo* successfully matured cumulus compared to cumulus from COCs that failed to mature *in vivo*, as well as compared to cumulus from COCs that matured successfully *in vitro*.
Embryo-specific fibronectin 1 transcript variant, as component of the extracellular matrix, exists in five isoforms due to alternative splicing and all have the same biological function (Goossens et al., 2009). A cumulus specific variant also exists for the bovine species and was already described in the same study as the here discussed embryo specific isoform (Goossens et al., 2009). Gene expression of isoforms differs between organs, between oocyte and cumulus and between maturation conditions (Goossens et al., 2009).
Cumulus cells secrete fibronectin during cumulus expansion (Relucenti et al., 2005; Sutovsky et al., 1995). Fibronectin is present in human follicular fluid, with a correlation to oocyte maturity and fertilizability (Tsuiki et al., 1988). A better oocyte quality was observed when its direct environment in the follicle contains higher concentrations of fibronectin (Honda et al., 2004). A paradoxal negative effect due to concurrence to the endogenous protein was reported when fibronectin is exogenously supplemented in fertilization medium (Thys et al., 2009).

The gene *FN1* was overexpressed in human cumulus from young patients compared to older ones (Al-Edani et al., 2014). Beside expression in the cumulus cells, fibronectin is also present in the extracellular matrix (Familiari et al., 1996; Thys et al., 2009). The fibronectin receptor protein integrin was described on sperm cells (Goossens et al., 2009; Thys et al., 2009), therefore it was suggested that it could be involved in sperm-oocyte binding (Diaz et al., 2007a; Hoshi et al., 1994; Martinez-Leon et al., 2015).

Different roles of fibronectin in oocyte maturation, oviductal pick-up and later embryo development were discussed in the literature (Familiari et al., 1996; Goossens et al., 2009; Relucenti et al., 2005).

Similar to the fact that maturation conditions may have influenced fibronectin expression in cumulus of the present study, bovine embryos produced *in vivo* presented a higher FN1 expression as those produced *in vitro* (Betteridge and Flechon, 1988; Goossens et al., 2007; Mohan et al., 2004; Takahashi et al., 2005).

α2-macroglobulin (A2M)

α2-macroglobulin was overexpressed in *in vivo* successfully matured cumulus compared to cumulus from COCs that failed to mature as well as compared to cumulus from COCs that matured successfully *in vitro*.

α2-macroglobulin is a protease inhibitor that prevents contact between protease and substrate via selective binding (Tayade et al., 2005). Due to its numerous possible binding partners, this protein is involved in a variety functions like hormonal control, angiogenesis, immune modulation and response, growth and differentiation of diverse cell types, signal transduction and embryo development (Tayade et al., 2005).

Presence of the protein in the ovary around time of ovulation was already reported (Gaddy-Kurten et al., 1989; Gaddy-Kurten and Richards, 1991; Vaughan and Vale, 1993). The synthesis of α2-macroglobulin was adjudged to the granulosa cells (Chung et al., 2009; Gaddy-Kurten et al., 1989; Gaddy-Kurten and Richards, 1991; Khan et al., 2016).

Gene expression of another α2-macroglobulin form was described for cumulus cells originating from mouse antral follicles (*A2mp*) (He et al., 2005). In humans, a derivate of α2-macroglobulin was detected in cumulus cells: the glycodelin interacting protein (GIP) (Chung et al., 2009). GIP is described in different species as a factor in the cumulus matrix that facilitate the fertilization (Fulop et al., 2003; Hizaki et al., 1999; Magier et al., 1990; Salustri et al., 2004; Zhang et al., 1995; Zhuo et al., 2001). Chung and coworkers suggested that cumulus is able to transform the α2-macroglobulin from surrounding environment to GIP.

GIP accumulates in the extracellular matrix and interacts with hyaluronic acid to retain there a factor that indirectly stimulates the spermatozoa-zona pellucida binding: glycodelin-C (Chung et al., 2009). This enables the cumulus cells to restore fertilizing capacity of sperm while they traverse the cumulus mass (Chung et al., 2009).

The detection of α2-macroglobulin in granulosa cells and the absence in cumulus cells, as reported in previous literature, has to be considered for the interpretation of the results of this study (Gaddy-Kurten et al., 1989; Gaddy-Kurten and Richards, 1991). A contamination of the cumulus samples with granulosa cells cannot be ruled out. Cumulus sampling is prone to contamination with mural granulosa cells attached to the COC. There may also be a difference in protein expression between cumulus directly surrounding oocyte and peripheral one (Hussein et al., 2005).

The fact that this protein shows a different expression between the different maturation conditions and outcomes shows, that α2-macroglobulin protein in somatic cells surrounding the oocyte may be influenced by maturation conditions. The described functions may play a role in further fertilization and development potential of matured oocytes.

CD9 antigen (CD9)

CD9 antigen was overexpressed in *in vivo* successfully matured cumulus compared to cumulus from COCs that failed to mature.

Maturation condition seems to impact CD9 expression as both, oocytes and cumulus cells, present an increased gene expression after IVM when a medium with expected positive impact on oocyte competence is used (Deb et al., 2012a). Within the COC, several descriptions of CD9 presence in association with the oocyte exist (Deb et al., 2012a; Ellerman et al., 2003; Ohnami et al., 2012; Okabe, 2014; Wen et al., 2007; Zhou et al., 2013), but only rare descriptions for cumulus cells are available (Assidi et al., 2010; Deb et al., 2012a). CD9 gene expression was also reported for bovine granulosa cells (Khan et al., 2016).

In oocytes, a reduced CD9 gene and protein expression can be associated with a reduced development potential (Wen et al., 2007; Zhou et al., 2013) as well as a reduced competence to bind sperms and being fertilized successfully (Zhou et al., 2013).

The identified CD9 is a cell-surface protein that is involved in gamete fusion during fertilization (Ellerman et al., 2003; Miyado et al., 2008; Ohnami et al., 2012). A lack of CD9 leads to impaired female fertility due to failure of sperm-egg fusion (Barraud-Lange et al., 2012; Ellerman et al., 2003; Kaji et al., 2000; Le Naour et al., 2000; Zhou et al., 2013). CD9 was also described as receptor for sperm (Jankovicova et al., 2015; Jegou et al., 2011). The

numerous mechanisms of function of CD9 around the fertilization process were already summarized in review papers (Jankovicova et al., 2015; Klinovska et al., 2014). The fusion of gametes is facilitated by CD9 containing vesicles transferred from the female gamete to the sperm (Miyado et al., 2008). CD9 can be observed in the perivitelline space, extracellular as well as in zona pellucida of oocytes (Ohnami et al., 2012). The CD9 antigens in the extracellular space are within extracellular vesicles, released from female gametes before fertilization and impact egg-sperm interaction (Ohnami et al., 2012). Extracellular vesicles seem to participate in sperm capacitation and acrosome reaction (Yanez-Mo et al., 2015). It is the female part that provides the CD9 as CD9 deficient sperm are still able to undergo successful fertilization in opposite to CD9 deficient COC (Barraud-Lange et al., 2012; Le Naour et al., 2000; Ohnami et al., 2012). Based on the fact that membrane fragment transfer occurs when sperms and cumulus cells are coincubated (Barraud-Lange et al., 2012), the presence of CD9 in cumulus cells may hypothetically influence sperm preparation before gametes meet. This hypothesis is strengthened by the observation of acrosome reactions on sperm while penetrating through the cumulus matrix of intact COCs, before reaching the zona pellucida (Bedford, 2011; Jin et al., 2011). Factors leading to this acrosome reaction are still not finally elucidated (Bedford, 2011) but cumulus proteins are already in older literature suspected to be involved (Tesarik et al., 1988).

Inter-alpha-trypsin inhibitor heavy chains H1 to H4 and pentraxin 3 influence post-ovulatory processes via their role in stability and expansion of the cumulus oophorus

Due to the previously reported role of Inter-alpha-trypsin inhibitor heavy chains H1 to H4 in cumulus stability, a logical role in oviductal pick-up of the COC is expected. These proteins, as well as pentraxin 3, are overexpressed in cumulus from *in vivo* successfully matured COCs compared to *in vitro* successfully matured COCs or COCs that failed to mature *in vivo*.

For Pentraxin 3, a similar negative effect on COC oviductal arrival was described for knockout mice, where only few oocytes reached the oviduct post ovulation. Cumulus cells were already lost before ovulation in several follicles. The disturbed cumulus seems to be an indirect cause for the impaired fertilization of COCs in these knockout females (Varani et al., 2002).

Inter-alpha-trypsin inhibitor impact also the journey of COC in the oviduct, with a negative effect on fertility (Hess et al., 1999).

Expression of proteins involved in post-ovulatory processes is altered by maturation conditions

The proteins discussed in this chapter are involved in the post-ovulatory processes up to fertilization. These proteins participate in diverse functions like oviductal retrieval, influence on fertilizing capacity of sperm or sperm-oocyte binding. They have an impact on the developmental potential of the COCs. The finding in our samples of proteins involved in post-ovulatory processes up to fertilization may explain why contact between cumulus cells and oocyte up to fertilization is so important to permit COC further development, according to previous literature (Fatehi et al., 2005). The different expression of these proteins leads to the hypothesis, that maturation condition alters the expression of proteins involved in post-ovulatory processes in cumulus cells. This may result in impaired oocyte developmental competence beyond maturation.

7.4.7 Proteins with influence on sperm

Proteins expressed in cumulus during maturation may influence attraction of the COC for spermatozoa and influence sperm motility. These proteins were all described in previous chapters, regarding other biological functions. Their additional function regarding the influence on sperm will be described in the following paragraphs.

Antithrombin III (SERPINC1)

Antithrombin III was upregulated after successful *in vivo* maturation compared to successful *in vitro* maturation or failure to reach MII under *in vivo* conditions.

Already described regarding its role in oxidative stress response, antithrombin III (Figure 21) may present another favourable effect when increased in cumulus of oocytes matured under optimal physiological conditions: the attraction of spermatozoa. In different studies, cumulus was already described as chemoattractive for sperm (Eisenbach and Giojalas, 2006; Sun et al., 2005). Compounds that may be in charge for this effect are antithrombin III (Lee et al., 1994) and hyaluronic acid (Sliwa, 1999). A stimulatory effect of antithrombin III on sperm motility was also described (Lee et al., 1994; Lee et al., 1992). Upregulation of antithrombin III in the cumulus mass of *in vivo* successfully matured COCs may result in a better chemoattractive function and higher motile sperm. This may be an explanation for better fertilization rates in COCs after *in vivo* maturation compared to *in vitro* maturation (Leibfried-Rutledge et al., 1987; Sanfins et al., 2015).

Complement component 3 (C3)

Complement component 3, like other proteins discussed in the complement and coagulation chapter, was upregulated after successful *in vivo* maturation compared to successful *in vitro* maturation or failure to reach MII under *in vivo* conditions.

The complement component 3 (Figure 27) also plays a role in gamete-binding previous to fertilization, with dose dependent effects on the process (Anderson et al., 1993; Anifandis et al., 2014).

Expression of proteins with influence on sperm is altered by maturation conditions

These two proteins have a chemo-attractive effect on sperm and a play role in gamete-binding. The higher expression in *in vivo* successfully matured COCs may be an explanation for the better fertilization capacity of COCs matured under *in vivo* conditions (Leibfried-Rutledge et al., 1987; Sanfins et al., 2015).

8 Conclusion

The presented results provide a first descriptive overview on the bovine cumulus proteome from single cumulus samples. The results give an insight into two different aspects: examination of the expressed proteins informs about influence of maturation, and the amount of quantifiable proteins show the potential of the novel method in single cumulus examination. The description if single proteins provide new hypothesis about potential influence of some proteins on biological function of cumulus.

Two maturation conditions and the different maturation outcomes for each were examined. The protein set examined here indicated some processes that seem to be affected under the non-physiological maturation conditions. The different expression of proteins, belonging to enriched biological pathways or individual proteins, provides novel information on the impact of maturation on the proteome. Several pathways were impacted:

-Complement and coagulation cascades
-Steroid biosynthesis
-Ovarian steroidogenesis
-N-Glycan biosynthesis
-ECM-receptor interaction
-Oxidative stress defence,
-Apoptosis
-Repair of DNA damage
-Gas transport
-Stability and expansion of the cumulus
-Post-ovulatory processes
-Influence on sperm

The maturation conditions impact the expression of proteins in cumulus, with influence on maturation success and further competence of the accompanying oocyte. Examination of individual proteins confirms the importance of the cumulus mass surrounding the bovine oocyte around maturation towards fertilization. These alterations in protein expression between maturation conditions give hints to the causes of the reduced developmental competence after *in vitro* maturation.

This information could be useful in order to improve the critical step of *ex vivo* maturation.

The amount of quantifiable proteins shows the potential of information in every single cumulus that the novel method was able to achieve. The method used show potential to screen single COCs in order to develop biomarkers sets for oocyte competence. Further studies on single COC level would allow correlating cumulus protein expression with outcome of the fertilized oocyte as it was already done with gene expression (Bunel et al., 2015; Kussano et al., 2015). The development of a set of protein markers for oocyte competence would be useful to improve the selection process for procedures like elective single embryo transfer.

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10 Annex

10.1 Annex 1: Medias composition

10.1.1 NaCl 0.9% with antibiotics

9g NaCl ad 1 l aqua bidest + 0.06g Penicillin + 0.1g Streptomycin

10.1.2 PBS working solution

PBS (Dulbecco's Phosphate Buffered Saline, Sigma D5773)

Step 1:

Stock Solution PBS (Dilution: 1:100 in Step 2)

		g/mol	G/L
D-Glucose (or D-Glucose Monohydrate) 1 g/L	Riedel de Haen 16301 Sigma Aldrich 49158	198.17	100.0g (109.8g)
Sodium pyruvate 0.4 mM	Sigma P3662	110	4.4g
$CaCl_2$ x 2 H_2O (or CaCl)	Sigma C7902	147	13.3g (10.04g)
Penicillin G (Kalium) * 50 – 100 IU/ml	Sigma PENK 1600 U/mg	327,48	6.0g
Streptomycin sulphate 50-100 µg/ml	Sigma S 6501 720 U/mg	145.,38	5g

* solve separately

Step 2:

Working Solution PBS

Aqua bidest	1 L
PBS Powder	9.65g
Stock Solution	10ml

Step 3:

add 5.6mg heparin (=1000 I.U) and 0.5g BSA to 500ml PBS working solution right before use.

10.1.3 TCM air

TCM 199 14.7g/L	Sigma M 2520	1.51g
Gentamycin sulphate (25) 50 µg/ml	Sigma G 3632	0.005g
Sodium pyruvat	Sigma P 3662	0.0022g
$NaHCO_3$	Riedel de Haen 31437 Sigma S5761	0.1g
*ad H_2O	Ampuwa Fresenius	100 ml
**BSA (FAF)	Sigma A 7030	0.1g

* solve separately

** add BSA after measuring the pH, then adjust to pH 7.2 using NaOH, filter through sterile filter, 266 mOsm

10.1.4 TCM + BSA (FAF)

TCM 199 14.7g/L	Sigma M 2520	1.51g
Gentamycin sulphate (25) 50µg/ml	Sigma G 3632	0.005g
Sodium pyruvat 110.044 g/mol 0.2mM (-0.5mM)	Sigma P 3662	0.0022g
$NaHCO_3$ 2.2g/Liter	Riedel de Haen 31437 Sigma S5761	0.22g
*ad H_2O	Ampuwa Fresenius	100 ml

**BSA (FAF) 1 (-4) mg/ml	Sigma A 7030	0.1g (0.4g)

* solve separately

** add BSA after measuring the pH

Stir in open beaker for about 1 hour until pH 7.4 is obtained, 285-290 mOsm

10.1.5 Hormones

1 aliquot (25µl) hormones (P.G. 600, Veterinaria)

- 400 I.U. PMSG + 200 I.U. hCG in dry substance
- solve in 1ml 0.9% NaCl

25 µl aliquots = 10 I.U. PMSG and 5 I.U. hCG /aliquot

10.1.6 PBS-PVA

100 ml PBS D8537
+ 0.0044g Sodium pyruvat (Sigma Aldrich P3662)
+ 0.1g D-Glucose (Sigma Aldrich 49158)
+ 0.01g PVA (Sigma Aldrich P8136)

10.1.7 Trypsin 1:5

20 µl Trypsin (Trypsin-EDTA solution, Sigma Aldrich T4174)
+ 80 µl PBS-PVA

11 Complete significant results

11.1 Matured: *in vitro* versus *in vivo*

						Successfully Matured: In Vivo		Successfully Matured: In Vitro			Successfully Matured: In Vivo		Successfully Matured: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|F1MN W4|F1MNW4_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Bos taurus GN=ITIH2 PE=4 SV=2	Successfully Matured: In Vivo	57	57	55.98	3030872.23	15294296.32	10428009.23	50035.35	153.77	17.82	0.50	12.86	0.23	0.0000	InVivo/SM>InVivo/FM
tr|Q5W1C4|Q5W1C4_BOVIN	Tumor necrosis factor, alpha-induced protein 6 OS=Bos taurus GN=tsg-6 PE=2 SV=1	Successfully Matured: In Vivo	15	15	14.80	9645479.45	5390328.89	3385296.15	26610.44	45.61	16.65	0.55	12.95	0.13	0.0000	InVivo/SM>InVivo/FM
sp|Q2UVX4|CO3_BOVIN	Complement C3 OS=Bos taurus GN=C3 PE=1 SV=2	Successfully Matured: In Vivo	59	59	57.54	4025457.84	1830152.55	1397632.92	53383.00	35.19	15.82	0.46	12.26	0.42	0.0000	InVivo/SM>InVivo/FM
sp|A6QLZ7|CRLD2_BOVIN;tr|D1Z306|D1Z306_BOVIN	Cysteine-rich secretory protein LCCL domain-containing 2 OS=Bos taurus GN=CRISPLD2 PE=2 SV=1 [sp|A6QLZ7|CRLD2_BOVIN,tr|D1Z306|D1Z306_BOVIN]	Successfully Matured: In Vivo	11	10	10.79	251522.33	136471.08	89668.40	1091.68	21.68	13.02	0.52	10.05	0.09	0.0000	InVivo/SM>InVivo/FM
tr|B8Y9S9|B8Y9S9_BOVIN	Embryo-specific fibronectin 1 transcript variant OS=Bos taurus GN=FN1 PE=2 SV=1	Successfully Matured: In Vivo	35	35	34.54	1370539.07	586456.93	470093.81	17221.83	56.17	14.76	0.41	10.63	0.62	0.0000	InVivo/SM>InVivo/FM
sp|P12763|FETUA_BOVIN_trunc	Alpha-2-HS-glycoprotein OS=Bos taurus GN=AHSG PE=1 SV=2	Successfully Matured: In Vivo	7	7	6.88	686449.13	579852.78	251018.32	2945.16	48.54	13.93	0.64	10.23	0.21	0.0000	InVivo/SM>InVivo/FM
sp|P49907|SEPP1_BOVIN	Selenoprotein P OS=Bos taurus GN=SEPP1 PE=2 SV=2	Successfully Matured: In Vivo	2	2	1.99	8331.97	8789.94	3096.92	1.73	10679.50	9.07	1.37	0.42	0.92	0.0000	InVivo/SM>InVivo/FM
tr|F1MGU7|F1MGU7_BOVIN	Fibrinogen gamma-B chain OS=Bos taurus GN=FGG PE=4 SV=1 [tr|F1MGU7|F1MGU7_BOVIN,tr|Q3SZZ9|Q3SZZ9_BOVIN]	Successfully Matured: In Vivo	29	29	28.50	5568359.09	2676561.06	1912678.53	15658.31	109.58	16.02	0.87	11.49	0.31	0.0000	InVivo/SM>InVivo/FM
tr|F1MAV0|F1MAV0_BOVIN	Fibrinogen beta chain OS=Bos taurus GN=FGB PE=4 SV=2	Successfully Matured: In Vivo	32	32	31.39	4718901.49	2389013.97	1630909.80	11514.92	82.86	15.86	0.85	11.63	0.21	0.0000	InVivo/SM>InVivo/FM
tr|Q9TS85|Q9TS85_BOVIN;tr|Q9BGU1|Q9BGU1_BOVIN	Histidine-rich GLYCOPROTEIN--FACTOR XIIIA substrate (Fragments) OS=Bos taurus PE=1 SV=1	Successfully Matured: In Vivo	3	1	2.99	37621.95	19031.90	12888.60	23.54	3574.14	11.13	0.50	0.93	2.08	0.0000	InVivo/SM>InVivo/FM
tr|G3N0S9|G3N0S9_BOVIN	Uncharacterized protein OS=Bos taurus GN=LOC515150 PE=4 SV=1	Successfully Matured: In Vivo	10	9	9.87	2986519.61	1818694.53	1045045.45	11306.37	126.59	15.36	0.91	10.69	0.42	0.0000	InVivo/SM>InVivo/FM
tr|A6QPP2|A6QPP2_BOVIN	SERPIND1 protein OS=Bos taurus GN=SERPIND1 PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.98	107819.47	42693.42	36932.66	2327.92	44.79	12.22	0.40	8.20	0.78	0.0000	InVivo/SM>InVivo/FM; InVivo/FM>InVitro/FM
tr|F1MMP5|F1MMP5_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Bos taurus GN=ITIH1 PE=4 SV=1	Successfully Matured: In Vivo	94	13	93.11	4168140.03	2888900.46	1472170.13	11285.25	283.95	15.69	0.85	9.98	0.95	0.0000	InVivo/SM>InVivo/FM
sp|P41361|ANT3_BOVIN;zz|ZZ_FGCZCont0237|	Antithrombin-III OS=Bos taurus GN=SERPINC1 PE=1 SV=2	Successfully Matured: In Vivo	15	15	14.89	292834.39	177417.92	105427.38	5346.46	21.34	13.17	0.49	10.14	0.49	0.0000	InVivo/SM>InVivo/FM
sp|P08728|K1C19_BOVIN	Keratin, type I cytoskeletal 19 OS=Bos taurus GN=KRT19 PE=2 SV=1	Successfully Matured: In Vivo	33	17	32.18	1281954.96	545782.68	464906.11	47523.15	10.76	14.69	0.41	12.33	0.36	0.0000	InVivo/SM>InVivo/FM
sp|A2I7N1|SPA35_BOVIN	Serpin A3-5 OS=Bos taurus GN=SERPINA3-5 PE=3 SV=1 [sp|A2I7N1|SPA35_BOVIN,tr|G8JKW7|G8JKW7_BOVIN]	Successfully Matured: In Vivo	9	1	8.79	51173.21	33922.39	18062.34	373.74	118.79	11.38	0.63	6.42	0.98	0.0000	InVivo/SM>InVivo/FM
tr|Q3ZBS7|Q3ZBS7_BOVIN	Uncharacterized protein OS=Bos taurus GN=VTN PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.87	563087.12	449881.08	203985.55	4626.43	59.60	13.68	0.82	9.76	0.46	0.0000	InVivo/SM>InVivo/FM
sp|P01966|HBA_BOVIN	Hemoglobin subunit alpha OS=Bos taurus GN=HBA PE=1 SV=2	Successfully Matured: In Vivo	15	15	14.61	16663176.62	11973473.07	5927932.93	41175.25	122.87	16.97	1.10	12.47	0.33	0.0000	InVivo/SM>InVivo/FM
sp|Q2KJF1|A1BG_BOVIN	Alpha-1B-glycoprotein OS=Bos taurus GN=A1BG PE=1 SV=1	Successfully Matured: In Vivo	5	5	4.88	55873.50	38002.57	19887.29	461.01	61.46	11.39	0.83	7.37	0.62	0.0000	InVivo/SM>InVivo/FM
sp|Q3T052|ITIH4_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Bos taurus GN=ITIH4 PE=1 SV=1 [sp|Q3T052|ITIH4_BOVIN,tr|F1MMD7|F1MMD7_BOVIN,tr|Q5EA67|Q5EA67_BOVIN]	Successfully Matured: In Vivo	5	5	4.86	94252.69	52470.39	32832.90	1567.63	81.73	12.04	0.50	7.11	1.21	0.0000	InVivo/SM>InVivo/FM
sp|P01044|KNG1_BOVIN	Kininogen-1 OS=Bos taurus GN=KNG1 PE=1 SV=1	Successfully Matured: In Vivo	5	5	4.96	87356.31	30506.84	30817.00	3685.26	14.18	12.00	0.45	9.28	0.58	0.0000	InVivo/SM>InVivo/FM
tr|F1MMK9|F1MMK9_BOVIN	Protein AMBP OS=Bos taurus GN=AMBP PE=4 SV=2	Successfully Matured: In Vivo	7	7	6.82	418683.59	314926.76	150066.12	2300.08	86.69	13.28	1.05	9.09	0.46	0.0000	InVivo/SM>InVivo/FM
tr|A5PJ77|A5PJ77_BOVIN	ECM1 protein OS=Bos taurus GN=ECM1 PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.99	104661.11	66481.91	37542.54	2189.89	27.86	12.08	0.68	8.79	0.59	0.0000	InVivo/SM>InVivo/FM
tr|F1MD77|F1MD77_BOVIN	Uncharacterized protein OS=Bos taurus GN=LAMC1 PE=4 SV=2	Successfully Matured: In Vivo	26	26	25.60	588606.59	353707.96	210127.12	22044.52	27.51	13.84	0.59	10.37	0.76	0.0000	InVivo/SM>InVivo/FM
tr|E1BAJ4|E1BAJ4_BOVIN	Uncharacterized protein OS=Bos taurus GN=FAM47E-STBD1 PE=4 SV=1	Successfully Matured: In Vivo	17	17	16.74	1803187.61	589782.27	671858.43	145084.19	6.65	15.06	0.30	13.11	0.46	0.0000	InVivo/SM>InVivo/FM
tr|E1BH06|E1BH06_BOVIN	Uncharacterized protein OS=Bos taurus GN=C4A PE=4 SV=2	Successfully Matured: In Vivo	18	3	17.83	74256.56	31383.54	25241.15	740.20	143.48	11.82	0.49	6.10	1.57	0.0001	InVivo/SM>InVivo/FM
tr|Q3T101|Q3T101_BOVIN	IGL@ protein OS=Bos taurus GN=IGL@ PE=1 SV=1	Successfully Matured: In Vivo	16	1	15.77	186842.77	63862.64	65714.15	9836.81	14.70	12.77	0.42	9.93	0.71	0.0001	InVivo/SM>InVivo/FM
sp|Q3T004|SAMP_BOVIN	Serum amyloid P-component OS=Bos taurus GN=APCS PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.98	11227.80	6029.57	3971.89	419.44	27.93	9.89	0.59	6.36	0.85	0.0001	InVivo/SM>InVivo/FM
tr|F1MYN5|F1MYN5_BOVIN	Uncharacterized protein OS=Bos taurus GN=FBLN1 PE=4 SV=2	Successfully Matured: In Vivo	19	19	18.76	4916292.37	2019309.44	1703016.34	167290.57	29.26	16.04	0.39	12.34	1.01	0.0001	InVivo/SM>InVivo/FM; InVivo/FM>InVitro/FM
tr|F1MU12|F1MU12_BOVIN;zz|ZZ_FGCZCont0125|	Keratin, type II cytoskeletal 8 OS=Bos taurus GN=KRT8 PE=3 SV=1	Successfully Matured: In Vivo	56	48	55.13	4791317.85	2569515.92	1745587.70	182375.58	12.96	15.95	0.56	13.42	0.50	0.0001	InVivo/SM>InVivo/FM
tr|F1N405|F1N405_BOVIN	Reticulon OS=Bos taurus GN=RTN4 PE=4 SV=1 [tr|F1N405|F1N405_BOVIN,tr|Q1RMR8|Q1RMR8_BOVIN]	Successfully Matured: In Vivo	8	8	7.85	505711.53	96680.53	220662.30	32823.70	2.38	13.81	0.19	12.95	0.17	0.0001	InVivo/SM>InVivo/FM
tr|G5E513|G5E513_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=1	Successfully Matured: In Vivo	3	2	2.97	40167.89	23517.41	13936.58	82.71	989.42	11.11	0.71	1.86	2.71	0.0001	InVivo/SM>InVivo/FM; InVitroFM>InVivoFM; InVitro/FM>InVitro/SM
sp|P00735|THRB_BOVIN	Prothrombin OS=Bos taurus GN=F2 PE=1 SV=2	Successfully Matured: In Vivo	24	24	23.59	778967.71	613170.19	287257.09	7146.88	22.80	13.98	0.85	11.12	0.19	0.0001	InVivo/SM>InVivo/FM
sp|P34955|A1AT_BOVIN	Alpha-1-antiproteinase OS=Bos taurus GN=SERPINA1 PE=1 SV=1	Successfully Matured: In Vivo	12	12	11.66	466765.78	277551.92	169770.02	19187.10	15.83	13.59	0.66	10.86	0.51	0.0001	InVivo/SM>InVivo/FM
sp|P56652|ITIH3_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H3 OS=Bos taurus GN=ITIH3 PE=1 SV=2	Successfully Matured: In Vivo	14	13	13.82	1176688.44	623083.12	409335.53	19750.17	62.54	14.56	0.53	9.93	1.34	0.0001	InVivo/SM>InVivo/FM
sp|Q5E9M1|PRAF3_BOVIN	PRA1 family protein 3 OS=Bos taurus GN=ARL6IP5 PE=2 SV=1	Successfully Matured: In Vivo	9	9	8.85	383323.89	120917.08	156554.32	22756.31	3.46	13.51	0.30	12.29	0.23	0.0001	InVivo/SM>InVivo/FM
sp|P02672|FIBA_BOVIN	Fibrinogen alpha chain OS=Bos taurus GN=FGA PE=1 SV=5 [sp|P02672|FIBA_BOVIN,tr|A5PJE3|A5PJE3_BOVIN]	Successfully Matured: In Vivo	30	30	29.43	3052108.94	1588131.29	1077497.02	47633.53	26.70	15.40	0.89	12.27	0.42	0.0001	InVivo/SM>InVivo/FM
sp|Q0VC16|MIA3_BOVIN	Melanoma inhibitory activity protein 3 OS=Bos taurus GN=MIA3 PE=2 SV=2 [sp|Q0VC16|MIA3_BOVIN,tr|G5E5L5|G5E5L5_BOVIN]	Successfully Matured: In Vivo	7	7	6.82	231415.02	59094.76	91553.83	19358.36	3.96	13.01	0.29	11.62	0.34	0.0001	InVivo/SM>InVivo/FM
tr|Q3SZQ8|Q3SZQ8_BOVIN;tr|Q32T06|Q32T06_BOVIN;tr|Q5J801|Q5J801_BOVIN	Endopin 2 OS=Bos taurus GN=SERPINA3-7 PE=2 SV=1 [tr|Q3SZQ8|Q3SZQ8_BOVIN,tr|Q5J801|Q5J801_BOVIN]	Successfully Matured: In Vivo	5	5	4.89	223443.42	141325.97	79895.50	6725.72	31.08	12.81	0.76	9.27	0.86	0.0001	InVivo/SM>InVivo/FM
sp|P81282|CSPG2_BOVIN;tr|F1MZ85|F1MZ85_BOVIN;tr|F1N615|F1N615_BOVIN	Versican core protein OS=Bos taurus GN=VCAN PE=1 SV=2 [sp|P81282|CSPG2_BOVIN,tr|F1MZ85|F1MZ85_BOVIN]	Successfully Matured: In Vivo	75	12	73.41	388434.22	175515.73	144586.90	22167.25	8.06	13.48	0.45	11.37	0.55	0.0002	InVivo/SM>InVivo/FM
tr|F1N076|F1N076_BOVIN	Uncharacterized protein OS=Bos taurus GN=CP PE=4 SV=2	Successfully Matured: In Vivo	9	9	8.80	96807.56	83824.23	35333.34	1253.56	73.43	11.86	0.92	7.41	1.19	0.0002	InVivo/SM>InVivo/FM
sp|Q29437|AOCX_BOVIN	Primary amine oxidase, liver isozyme OS=Bos taurus PE=1 SV=1	Successfully Matured: In Vivo	2	2	1.96	8912.17	5242.32	3127.52	171.98	68.64	9.59	0.80	4.72	1.50	0.0002	InVivo/SM>InVivo/FM; InVivo/FM>InVitro/FM; InVitro/SM>InVitro/FM
sp|P17690|APOH_BOVIN	Beta-2-glycoprotein 1 OS=Bos taurus GN=APOH PE=1 SV=4	Successfully Matured: In Vivo	4	4	3.98	54114.27	59340.84	20466.44	1229.21	56.75	11.20	0.94	7.00	1.15	0.0002	InVivo/SM>InVivo/FM; InVivo/FM>InVitro/FM
sp|P79132|CAV1_BOVIN	Caveolin-1 OS=Bos taurus GN=CAV1 PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.91	75425.68	77215.77	28251.69	830.50	54.32	11.43	1.13	7.77	0.64	0.0002	InVivo/SM>InVivo/FM
tr|Q08DC0|Q08DC0_BOVIN	Serpin peptidase inhibitor, clade E (Nexin, plasminogen activator inhibitor type 1), member 2 OS=Bos taurus GN=SERPINE2 PE=2 SV=1 [tr|Q08DC0|Q08DC0_BOVIN,tr|Q8HZY1|Q8HZY1_BOVIN]	Successfully Matured: In Vivo	24	24	23.44	4081567.21	1446102.52	1522891.46	343974.64	6.80	15.86	0.39	13.86	0.60	0.0003	InVivo/SM>InVivo/FM
tr|A6H7D3|A6H7D3_BOVIN;tr|F6S1Q0|F6S1Q0_BOVIN;zz|ZZ_FGCZCont0035|;zz|ZZ_FGCZCont0086|	KRT18 protein (Fragment) OS=Bos taurus GN=KRT18 PE=2 SV=1 [tr|A6H7D3|A6H7D3_BOVIN,tr|F6S1Q0|F6S1Q0_BOVIN]	Successfully Matured: In Vivo	24	18	23.41	1700593.63	1167995.95	630994.01	59945.43	13.78	14.82	0.78	12.31	0.52	0.0003	InVivo/SM>InVivo/FM
tr|A6QM09|A6QM09_BOVIN	Putative uncharacterized protein OS=Bos taurus PE=2 SV=1	Successfully Matured: In Vivo	17	2	16.69	32960.08	29759.85	12202.08	410.98	38.74	10.65	1.14	7.32	0.56	0.0004	InVivo/SM>InVivo/FM
tr|F1MYA8|F1MYA8_BOVIN	Uncharacterized protein OS=Bos taurus GN=ATP1A1 PE=3 SV=1	Successfully Matured: In Vivo	5	5	4.95	136532.46	58036.24	53360.46	8855.84	5.00	12.43	0.49	10.86	0.35	0.0004	InVivo/SM>InVivo/FM
sp|P19660|CTHL2_BOVIN	Cathelicidin-2 OS=Bos taurus GN=CATHL2 PE=1 SV=2	Successfully Matured: In Vivo	2	2	1.99	12295.52	10673.19	4454.32	28.90	485.25	9.64	1.24	2.59	2.40	0.0004	InVivo/SM>InVivo/FM
sp|Q3MHN2|CO9_BOVIN	Complement component C9 OS=Bos taurus GN=C9 PE=2 SV=1	Successfully Matured: In Vivo	6	6	5.89	166034.10	103583.26	62127.98	10881.04	10.30	12.57	0.59	10.19	0.70	0.0004	InVivo/SM>InVivo/FM
sp|P67810|SC11A_BOVIN;tr|Q9BDH8|Q9BDH8_BOVIN	Signal peptidase complex catalytic subunit SEC11A OS=Bos taurus GN=SEC11A PE=2 SV=1	Successfully Matured: In Vivo	5	5	4.84	161226.50	34748.36	70887.02	17771.49	2.34	12.67	0.20	11.81	0.26	0.0004	InVivo/SM>InVivo/FM
tr|F1MEG3|F1MEG3_BOVIN	Uncharacterized protein OS=Bos taurus GN=LAMA1 PE=4 SV=2	Successfully Matured: In Vivo	42	42	41.15	714720.60	536740.68	271564.29	29678.37	10.68	13.94	0.76	11.74	0.39	0.0004	InVivo/SM>InVivo/FM
tr|F1MY84|F1MY84_BOVIN	Uncharacterized protein OS=Bos taurus GN=CSPG4 PE=4 SV=2	Successfully Matured: In Vivo	30	29	29.76	1036078.48	444562.25	388317.34	138522.98	7.23	14.47	0.42	12.29	0.76	0.0005	InVivo/SM>InVivo/FM
sp|P02070|HBB_BOVIN;tr|D4QBB4|D4QBB4_BOVIN;zz|ZZ_FGCZCont0174|	Hemoglobin subunit beta OS=Bos taurus GN=HBB PE=1 SV=1 [sp|P02070|HBB_BOVIN,tr|D4QBB4|D4QBB4_BOVIN]	Successfully Matured: In Vivo	30	6	29.39	2076138.14	1672666.35	770011.96	48526.98	20.73	14.95	0.89	12.04	0.75	0.0005	InVivo/SM>InVivo/FM

						Successfully Matured: In Vivo		Successfully Matured: In Vitro			Successfully Matured: In Vivo		Successfully Matured: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|G3X6N3|G3X6N3_BOVIN;sp|P24627|TRFL_BOVIN;tr|B3VTM3|B3VTM3_BOVIN;tr|B9VPZ5|B9VPZ5_BOVIN;tr|C7FE01|C7FE01_BOVIN;tr|E1BI82|E1BI82_BOVIN;tr|Q2HJF0|Q2HJF0_BOVIN;tr|Q6LBN7|Q6LBN7_BOVIN;tr|Q8MII0|Q8MII0_BOVIN	Serotransferrin OS=Bos taurus GN=TF PE=4 SV=1	Successfully Matured: In Vivo	32	31	31.71	2173835.55	1128818.24	780987.57	167314.29	16.30	15.16	0.60	11.92	1.15	0.0005	InVivo/SM>InVivo/FM
sp|O46375|TTHY_BOVIN	Transthyretin OS=Bos taurus GN=TTR PE=1 SV=1	Successfully Matured: In Vivo	5	5	4.93	845318.05	297381.25	317596.06	84636.14	6.36	14.29	0.35	12.30	0.73	0.0006	InVivo/SM>InVivo/FM; InVitro/FM>InVivo/FM
sp|P42891|ECE1_BOVIN	Endothelin-converting enzyme 1 OS=Bos taurus GN=ECE1 PE=1 SV=2	Successfully Matured: In Vivo	6	6	5.85	109035.83	63745.83	42134.60	4526.08	6.61	12.13	0.65	10.37	0.30	0.0006	InVivo/SM>InVivo/FM
tr|E1BJK2|E1BJK2_BOVIN	Uncharacterized protein OS=Bos taurus GN=TUBB1 PE=3 SV=1	Successfully Matured: In Vivo	13	1	12.89	6050.40	4425.45	2161.31	126.11	107.28	8.95	1.35	1.27	2.83	0.0006	InVivo/SM>InVivo/FM
sp|P06868|PLMN_BOVIN	Plasminogen OS=Bos taurus GN=PLG PE=1 SV=2 [sp|P06868|PLMN_BOVIN;tr|E1B726|E1B726_BOVIN]	Successfully Matured: In Vivo	3	3	2.97	25813.50	29530.37	9724.48	61.13	444.77	10.45	0.93	3.11	2.86	0.0006	InVivo/SM>InVivo/FM
tr|F1N1E5|F1N1E5_BOVIN	Thioredoxin domain-containing protein 11 OS=Bos taurus GN=TXNDC11 PE=4 SV=1	Successfully Matured: In Vivo	3	3	2.99	9013.22	17011.77	3750.98	56.02	359.74	8.42	1.72	1.10	2.47	0.0006	InVivo/SM>InVivo/FM
sp|P04272|ANXA2_BOVIN;tr|REV_F1MR60|REV_F1MR60_BOVIN;tr|REV_Q29RB5|REV_TFP11_BOVIN	Annexin A2 OS=Bos taurus GN=ANXA2 PE=1 SV=2	Successfully Matured: In Vivo	26	26	25.67	2433205.01	496580.33	1062921.40	320850.38	2.39	15.38	0.21	14.49	0.30	0.0006	InVivo/SM>InVivo/FM
tr|E1BDK6|E1BDK6_BOVIN;tr|REV_E1BJ09|REV_E1BJ09_BOVIN	Uncharacterized protein OS=Bos taurus GN=LAMB2 PE=4 SV=2	Successfully Matured: In Vivo	25	25	24.59	564777.98	444938.66	209845.92	28114.19	18.41	13.67	0.81	10.69	0.93	0.0007	InVivo/SM>InVivo/FM
tr|F1ME65|F1ME65_BOVIN	Uncharacterized protein OS=Bos taurus GN=CKAP4 PE=4 SV=1	Successfully Matured: In Vivo	47	47	46.12	11684134.87	2815242.47	5227394.44	1226776.44	2.21	16.94	0.24	16.15	0.23	0.0007	InVivo/SM>InVivo/FM
tr|Q1RMH5|Q1RMH5_BOVIN	C1QC protein (Fragment) OS=Bos taurus GN=C1QC PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.93	125823.15	83452.47	44348.85	952.37	151.11	12.03	1.29	6.56	1.88	0.0007	InVivo/SM>InVivo/FM
tr|F1MX87|F1MX87_BOVIN	Uncharacterized protein OS=Bos taurus GN=C8A PE=4 SV=1 [tr|F1MX87|F1MX87_BOVIN;tr|Q1JPD0|Q1JPD0_BOVIN;tr|Q2KIH5|Q2KIH5_BOVIN]	Successfully Matured: In Vivo	2	2	1.99	21021.65	21073.30	7810.98	233.06	91.96	10.25	0.99	5.28	1.85	0.0007	InVivo/SM>InVivo/FM
tr|A3KMW5|A3KMW5_BOVIN	C1QTNF1 protein OS=Bos taurus GN=C1QTNF1 PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.87	56338.43	45915.57	21128.65	3363.02	16.16	11.38	0.77	8.47	0.97	0.0008	InVivo/SM>InVivo/FM
sp|Q3SYT8|NCPR_BOVIN	NADPH--cytochrome P450 reductase OS=Bos taurus GN=POR PE=2 SV=3 [sp|Q3SYT8|NCPR_BOVIN;tr|A5D9D3|A5D9D3_BOVIN]	Successfully Matured: In Vivo	34	34	33.58	1948936.73	911212.81	783639.55	153017.77	4.27	15.08	0.48	13.68	0.36	0.0008	InVivo/SM>InVivo/FM
tr|B0JYP6|B0JYP6_BOVIN	IGK protein OS=Bos taurus GN=IGK PE=2 SV=1 [tr|B0JYP6|B0JYP6_BOVIN;tr|F1MH40|F1MH40_BOVIN;tr|F1MZ96|F1MZ96_BOVIN;tr|Q05B55|Q05B55_BOVIN]	Successfully Matured: In Vivo	7	6	6.94	222831.48	190342.53	82245.78	11205.18	31.60	12.65	1.03	8.74	1.32	0.0008	InVivo/SM>InVivo/FM
tr|Q32LF7|Q32LF7_BOVIN	UDP-GalbetaGlcNAc beta 1,4-galactosyltransferase, polypeptide 4 OS=Bos taurus GN=B4GALT4 PE=2 SV=1	Successfully Matured: In Vivo	9	9	8.77	369396.80	59102.39	144393.09	58415.41	3.95	13.50	0.15	11.98	0.64	0.0008	InVivo/SM>InVivo/FM
tr|F1MPR3|F1MPR3_BOVIN;tr|F1MGE7|F1MGE7_BOVIN	Uncharacterized protein OS=Bos taurus GN=ATP2A2 PE=3 SV=2	Successfully Matured: In Vivo	36	30	35.38	930163.72	236518.04	403541.50	123881.34	2.51	14.41	0.23	13.47	0.33	0.0008	InVivo/SM>InVivo/FM
zz|ZZ_FGCZCont0243|	gi|12697269|sp|Q99456|K1C1_HUMAN KERATIN, TYPE I CYTOSKELETAL 12 (CYTO	Successfully Matured: In Vivo	11	1	10.72	36499.75	22538.34	13238.47	1656.26	18.38	10.92	1.00	8.05	0.75	0.0009	InVivo/SM>InVivo/FM
tr|G3N2D7|G3N2D7_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=IGLL1 PE=4 SV=1	Successfully Matured: In Vivo	2	2	1.90	680116.77	279136.96	235836.96	22800.24	28.11	14.04	0.46	10.07	1.67	0.0009	InVivo/SM>InVivo/FM
tr|Q6JE76|Q6JE76_BOVIN	PDFT1 protein OS=Bos taurus GN=fdft1 PE=2 SV=1	Successfully Matured: In Vivo	6	6	5.91	125797.17	52644.07	46797.89	13517.79	7.70	12.37	0.39	10.07	0.93	0.0009	
tr|G3N0V0|G3N0V0_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=1	Successfully Matured: In Vivo	11	11	10.78	3149870.65	1989594.86	1136665.62	164555.38	22.25	15.40	0.94	12.00	1.15	0.0009	InVivo/SM>InVivo/FM
tr|Q70E76|Q70E76_BOVIN	Alpha2,3-sialyltransferase OS=Bos taurus GN=ST3GAL4V PE=2 SV=1	Successfully Matured: In Vivo	14	14	13.87	1134682.51	590881.66	419930.38	84140.28	9.79	14.52	0.56	12.07	0.92	0.0009	InVivo/SM>InVivo/FM; InVivo/FM>InVitro/FM
tr|Q1RMN9|Q1RMN9_BOVIN	Cab-binding protein alpha-like OS=Bos taurus GN=LOC510860 PE=2 SV=1	Successfully Matured: In Vivo	6	1	5.87	2398.77	2006.11	875.65	38.32	63.53	8.17	0.87	3.48	1.87	0.0010	InVivo/SM>InVivo/FM
tr|E1BLR9|E1BLR9_BOVIN	Uncharacterized protein OS=Bos taurus GN=CPD PE=4 SV=2	Successfully Matured: In Vivo	13	13	12.65	219264.34	92922.89	89784.14	26777.94	3.69	12.92	0.40	11.61	0.42	0.0010	
tr|A1A3Z1|A1A3Z1_BOVIN	Low density lipoprotein receptor-related protein 8 OS=Bos taurus GN=LRP8 PE=2 SV=1	Successfully Matured: In Vivo	17	16	16.80	509851.57	511198.16	189971.38	9595.49	65.25	13.19	1.47	9.22	0.95	0.0010	InVivo/SM>InVivo/FM
tr|F1MLW8|F1MLW8_BOVIN	Uncharacterized protein OS=Bos taurus PE=4 SV=2	Successfully Matured: In Vivo	16	3	15.78	71204.71	40233.95	25109.25	1921.19	37.31	11.67	0.79	7.59	1.62	0.0010	InVivo/SM>InVivo/FM
sp|Q3SZR3|A1AG_BOVIN	Alpha-1-acid glycoprotein OS=Bos taurus GN=ORM1 PE=2 SV=1 [sp|Q3SZR3|A1AG_BOVIN;tr|Q5GN72|Q5GN72_BOVIN]	Successfully Matured: In Vivo	7	7	6.87	211655.72	132747.02	76281.36	10866.57	22.72	12.72	0.83	9.24	1.30	0.0010	InVivo/SM>InVivo/FM
sp|P62157|CALM_BOVIN	Calmodulin OS=Bos taurus GN=CALM PE=1 SV=2	Successfully Matured: In Vivo	10	9	9.83	869055.43	463761.97	340607.14	79730.56	5.48	14.27	0.49	12.54	0.59	0.0010	
tr|Q2KII3|Q2KII3_BOVIN	Hepatitis A virus cellular receptor 1 N-terminal domain containing protein OS=Bos taurus GN=MGC137099 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.97	121783.39	83112.94	43759.93	4939.38	34.69	12.01	1.20	8.21	1.20	0.0010	InVivo/SM>InVivo/FM
tr|G1K238|G1K238_BOVIN;tr|F1MZU6|F1MZU6_BOVIN;tr|F1N474|F1N474_BOVIN	Collagen alpha-1(IV) chain OS=Bos taurus GN=COL4A1 PE=4 SV=2	Successfully Matured: In Vivo	2	1	1.97	61025.47	43924.97	23513.36	3438.79	8.34	11.49	0.74	9.50	0.49	0.0010	InVivo/SM>InVivo/FM
tr|Q3SZI3|Q3SZI3_BOVIN	Chromosome 5 open reading frame 15 ortholog OS=Bos taurus GN=C7H5ORF15 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.98	13102.15	8370.63	4836.71	1189.66	13.50	10.02	0.62	7.00	1.19	0.0010	
sp|Q3MHN5|VTDB_BOVIN	Vitamin D-binding protein OS=Bos taurus GN=GC PE=2 SV=1 [sp|Q3MHN5|VTDB_BOVIN;tr|F1N5M2|F1N5M2_BOVIN;tr|I7CT57|I7CT57_BOVIN]	Successfully Matured: In Vivo	5	5	4.91	53455.78	34872.24	19455.99	1135.33	19.11	11.23	1.14	8.57	0.40	0.0011	InVivo/SM>InVivo/FM
sp|A2VE10|CASC4_BOVIN	Protein CASC4 OS=Bos taurus GN=CASC4 PE=2 SV=1	Successfully Matured: In Vivo	5	5	4.77	110024.45	33520.85	45005.41	14955.06	3.41	12.26	0.32	10.98	0.49	0.0012	InVivo/SM>InVivo/FM
tr|A4IFB8|A4IFB8_BOVIN	IPO4 protein OS=Bos taurus GN=IPO4 PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.95	311565.64	180020.13	110821.79	12513.13	27.76	13.07	1.00	9.40	1.35	0.0012	InVivo/SM>InVivo/FM
sp|P07995|INHBA_BOVIN	Inhibin beta A chain OS=Bos taurus GN=INHBA PE=1 SV=2	Successfully Matured: In Vivo	9	9	8.65	77955.45	48020.24	28033.89	5231.28	23.17	11.66	1.03	8.04	1.30	0.0012	
tr|F1N7Q1|F1N7Q1_BOVIN	Uncharacterized protein OS=Bos taurus GN=PDE8A PE=3 SV=2	Successfully Matured: In Vivo	2	2	1.85	1911.71	1227.13	721.87	143.38	9.86	8.04	0.77	5.74	0.73	0.0013	
tr|A7Z066|A7Z066_BOVIN;tr|Q2EGT5|Q2EGT5_BOVIN	Canx protein OS=Bos taurus GN=canx PE=2 SV=1	Successfully Matured: In Vivo	31	30	30.23	5523218.53	1357386.34	2515536.74	571350.48	2.08	16.19	0.25	15.47	0.23	0.0013	
sp|P02453|CO1A1_BOVIN	Collagen alpha-1(I) chain OS=Bos taurus GN=COL1A1 PE=1 SV=3	Successfully Matured: In Vivo	3	3	2.99	93616.34	47853.31	34644.07	8192.12	9.63	12.00	0.65	9.53	0.98	0.0015	InVivo/SM>InVivo/FM
tr|Q0V8K9|Q0V8K9_BOVIN	Solute carrier family 29 (Nucleoside transporters), member 1 (Fragment) OS=Bos taurus GN=SLC29A1 PE=2 SV=1 [tr|Q0V8K9|Q0V8K9_BOVIN;tr|Q3ZC83|Q3ZC83_BOVIN]	Successfully Matured: In Vivo	4	4	3.88	240565.20	69264.11	99150.64	51176.12	3.24	13.05	0.27	11.77	0.55	0.0015	InVivo/SM>InVivo/FM
sp|Q05B45|T120A_BOVIN	Transmembrane protein 120A OS=Bos taurus GN=TMEM120A PE=2 SV=1	Successfully Matured: In Vivo	14	14	13.61	804164.65	451020.46	319351.64	71264.78	5.08	14.17	0.54	12.55	0.57	0.0017	InVivo/SM>InVivo/FM
tr|D1Z308|D1Z308_BOVIN;tr|G5E6M0|G5E6M0_BOVIN;tr|P9QD97|P9QD97_BOVIN;tr|P9QDG2|P9QDG2_BOVIN	Periostin OS=Bos taurus GN=POSTN PE=2 SV=1 [tr|D1Z308|D1Z308_BOVIN;tr|G5E6M0|G5E6M0_BOVIN;tr|P9QD97|P9QD97_BOVIN;tr|P9QDG2|P9QDG2_BOVIN]	Successfully Matured: In Vivo	8	1	7.95	6912.10	7192.31	2573.98	51.69	185.99	8.87	1.62	2.09	2.86	0.0017	InVivo/SM>InVivo/FM
tr|A6QQP4|A6QQP4_BOVIN	SCARB2 protein OS=Bos taurus GN=SCARB2 PE=2 SV=1	Successfully Matured: In Vivo	5	5	4.89	352039.46	186960.33	142124.37	37856.59	4.39	13.35	0.52	11.89	0.48	0.0018	
tr|D4QBB3|D4QBB3_BOVIN	Hemoglobin beta OS=Bos taurus GN=HBB PE=3 SV=1	Successfully Matured: In Vivo	26	2	25.49	1057453.17	1203632.89	399201.84	7797.29	145.26	13.63	1.94	9.20	0.94	0.0018	InVivo/SM>InVivo/FM; InVivo/FM>InVitro/FM
tr|Q17QL5|Q17QL5_BOVIN	Transmembrane protein 30A OS=Bos taurus GN=TMEM30A PE=2 SV=1	Successfully Matured: In Vivo	9	9	8.79	87195.74	65657.56	33255.10	9317.31	10.15	11.88	0.65	9.31	1.08	0.0018	InVivo/SM>InVivo/FM
sp|Q3SZV7|HEMO_BOVIN	Hemopexin OS=Bos taurus GN=HPX PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.95	72534.36	31843.66	24707.45	566.38	156.30	11.80	0.47	5.40	3.11	0.0019	InVivo/SM>InVivo/FM
sp|Q5ZBE9|NSDHL_BOVIN	Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating OS=Bos taurus GN=NSDHL PE=2 SV=1	Successfully Matured: In Vivo	6	6	5.97	128153.40	46849.21	51895.61	17767.98	3.77	12.40	0.36	11.00	0.59	0.0019	InVivo/SM>InVivo/FM
tr|F1N3G6|F1N3G6_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=ATP1C PE=4 SV=2	Successfully Matured: In Vivo	13	13	12.77	233752.09	80848.91	92921.20	41585.79	4.14	13.01	0.31	11.42	0.72	0.0020	
sp|A6QPQ2|SPA38_BOVIN	Serpin A3-8 OS=Bos taurus GN=SERPINA3-8 PE=2 SV=1	Successfully Matured: In Vivo	5	2	4.77	28195.92	30099.27	10518.03	101.28	279.59	10.36	1.41	3.46	3.17	0.0022	InVivo/SM>InVivo/FM
sp|Q2TBI0|LBP_BOVIN	Lipopolysaccharide-binding protein OS=Bos taurus GN=LBP PE=2 SV=1 [sp|Q2TBI0|LBP_BOVIN;tr|F1MNN7|F1MNN7_BOVIN]	Successfully Matured: In Vivo	3	3	2.91	28758.70	41648.64	11418.67	591.97	53.42	10.10	1.44	6.48	1.13	0.0022	InVivo/SM>InVivo/FM
sp|P17697|CLUS_BOVIN	Clusterin OS=Bos taurus GN=CLU PE=1 SV=1	Successfully Matured: In Vivo	4	4	3.91	51702.06	28268.36	19122.57	1881.11	10.43	11.28	1.00	9.14	0.42	0.0022	
sp|Q3SZI6|RPN2_BOVIN	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 OS=Bos taurus GN=RPN2 PE=2 SV=1	Successfully Matured: In Vivo	17	17	16.91	1420655.86	541763.49	620248.67	173519.54	2.61	14.80	0.38	13.87	0.27	0.0022	InVivo/SM>InVivo/FM
tr|F1N7Q7|F1N7Q7_BOVIN	Collagen alpha-2(IV) chain (Fragment) OS=Bos taurus GN=COL4A2 PE=4 SV=2	Successfully Matured: In Vivo	4	4	3.75	74493.47	78966.92	28558.63	1778.39	22.41	11.32	1.25	8.72	0.42	0.0022	InVivo/SM>InVivo/FM

						Successfully Matured: In Vivo		Successfully Matured: In Vitro			Successfully Matured: In Vivo		Successfully Matured: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|A6QP98|A6QP98_BOVIN	LOC529366 protein (Fragment) OS=Bos taurus GN=LOC529366 PE=2 SV=1 [tr|A6QP98|A6QP98_BOVIN,tr|E1BMC6|E1BMC6_BOVIN]	Successfully Matured: In Vivo	8	8	7.81	120618.76	103855.50	45636.37	6151.41	15.29	12.00	1.07	9.45	0.72	0.0022	InVivo/SM>InVivo/FM
tr|F1N7T2|F1N7T2_BOVIN	Uncharacterized protein OS=Bos taurus GN=MAN2A1 PE=4 SV=2	Successfully Matured: In Vivo	3	3	2.99	57168.04	27476.27	22362.06	8767.32	5.26	11.56	0.46	9.73	0.82	0.0024	InVivo/SM>InVivo/FM
sp|Q3SIH1|A2MG_BOVIN	Alpha-2-macroglobulin OS=Bos taurus GN=A2M PE=1 SV=2	Successfully Matured: In Vivo	16	5	15.70	126278.38	91994.17	46420.86	6507.18	19.57	12.16	0.88	8.79	1.49	0.0024	InVivo/SM>InVivo/FM
sp|Q28198|TPA_BOVIN	Tissue-type plasminogen activator OS=Bos taurus GN=PLAT PE=2 SV=1	Successfully Matured: In Vivo	6	6	5.96	117879.35	58178.71	45003.28	28953.45	6.58	12.26	0.53	9.73	1.19	0.0024	InVivo/SM>InVivo/FM
sp|Q3SZE3|LPP3_BOVIN	Lipid phosphate phosphohydrolase 3 OS=Bos taurus GN=PPAP2B PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.97	15402.13	9726.34	6306.87	1877.98	4.37	10.19	0.58	8.77	0.46	0.0027	InVivo/SM>InVivo/FM
tr|E1B731|E1B731_BOVIN	Uncharacterized protein OS=Bos taurus GN=TMEM165 PE=4 SV=1	Successfully Matured: In Vivo	2	2	1.99	209387.71	70107.34	78385.91	42313.14	6.43	12.90	0.32	10.46	1.24	0.0028	InVivo/SM>InVivo/FM
tr|A3KN04|A3KN04_BOVIN	RPN1 protein OS=Bos taurus GN=RPN1 PE=2 SV=1	Successfully Matured: In Vivo	34	28	33.46	3157131.77	1028480.74	1448943.23	309614.30	2.09	15.62	0.32	14.91	0.20	0.0030	
tr|A5D7G6|A5D7G6_BOVIN	ST3B protein OS=Bos taurus GN=ST3B PE=2 SV=1	Successfully Matured: In Vivo	9	9	8.78	243729.94	93573.43	105097.36	38868.36	2.76	13.04	0.36	12.01	0.42	0.0030	InVivo/SM>InVivo/FM
sp|A6QPY0|OST48_BOVIN	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit OS=Bos taurus GN=DDOST PE=2 SV=2 [sp|A6QPY0|OST48_BOVIN,tr|F1N632|F1N632_BOVIN]	Successfully Matured: In Vivo	14	14	13.74	1446725.48	391875.35	655712.48	214329.70	2.14	14.85	0.26	14.08	0.32	0.0032	
sp|Q2NL17|CLPT1_BOVIN	Cleft lip and palate transmembrane protein 1 homolog OS=Bos taurus GN=CLPTM1 PE=2 SV=1	Successfully Matured: In Vivo	6	6	5.96	33116.27	22577.74	12555.13	2022.79	9.62	10.88	0.78	8.57	0.98	0.0033	InVivo/SM>InVivo/FM
sp|Q2KJH6|SERPH_BOVIN	Serpin H1 OS=Bos taurus GN=SERPINH1 PE=2 SV=1	Successfully Matured: In Vivo	54	54	53.40	17664963.44	5852353.23	7869715.00	2221166.40	2.34	17.33	0.34	16.49	0.31	0.0034	
tr|A6QR14|A6QR14_BOVIN	DHCR24 protein OS=Bos taurus GN=DHCR24 PE=2 SV=1	Successfully Matured: In Vivo	11	11	10.82	726995.51	331395.36	278815.08	133013.22	6.02	14.12	0.42	11.89	1.15	0.0036	InVivo/SM>InVivo/FM
tr|A6QNW7|A6QNW7_BOVIN	CD5L protein OS=Bos taurus GN=CD5L PE=2 SV=1 [tr|A6QNW7|A6QNW7_BOVIN,tr|F1N514|F1N514_BOVIN]	Successfully Matured: In Vivo	7	7	6.88	45741.45	30077.00	16843.33	2964.05	14.82	11.09	1.09	8.31	1.09	0.0037	InVivo/SM>InVivo/FM; InVitro/FM>InVitro/SM; InVitro/FM>InVivo/FM
zz|ZZ_FGCZCont0047|	gi|181994 (381419) hair type I acidic keratin [Homo sapiens] gi|16687 [zz|ZZ_FGCZCont0047|,zz|ZZ_FGCZCont0144|]	Successfully Matured: In Vivo	7	1	6.77	8161.13	2127.52	2915.57	1419.34	9.62	9.67	0.26	6.12	1.95	0.0037	InVivo/SM>InVivo/FM
tr|Q3ZBX0|Q3ZBX0_BOVIN	Basigin OS=Bos taurus GN=BSG PE=2 SV=1	Successfully Matured: In Vivo	11	11	10.90	6858620.29	3109190.92	2669612.92	1105475.86	5.31	16.33	0.53	14.45	0.90	0.0037	InVivo/SM>InVivo/FM
tr|E1BEI2|E1BEI2_BOVIN	Uncharacterized protein OS=Bos taurus GN=SPCS2 PE=4 SV=1	Successfully Matured: In Vivo	9	9	8.71	231927.14	82871.46	101060.85	25551.41	2.60	12.99	0.41	12.05	0.32	0.0039	
sp|Q9N2I2|IPSP_BOVIN	Plasma serine protease inhibitor OS=Bos taurus GN=SERPINA5 PE=1 SV=1	Successfully Matured: In Vivo	2	2	1.93	4233.34	5786.14	1683.80	338.11	27.99	8.04	1.68	1.47	3.27	0.0040	InVivo/SM>InVivo/FM
sp|A7E3W2|LG3BP_BOVIN	Galectin-3-binding protein OS=Bos taurus GN=LGALS3BP PE=1 SV=1	Successfully Matured: In Vivo	18	18	17.70	731199.23	537830.84	298160.97	85255.02	4.94	14.00	0.68	12.48	0.53	0.0043	InVivo/SM>InVivo/FM
tr|E1BP48|E1BP48_BOVIN	Uncharacterized protein OS=Bos taurus GN=KHDRBS2 PE=4 SV=2	Successfully Matured: In Vivo	2	1	1.90	21345.17	31498.72	8427.12	76.04	219.51	9.60	1.82	4.36	2.37	0.0044	InVivo/SM>InVivo/FM; InVitro/FM>InVitro/SM
sp|Q29RQ1|CO7_BOVIN	Complement component C7 OS=Bos taurus GN=C7 PE=2 SV=1 [sp|Q29RQ1|CO7_BOVIN,tr|F1N0451|F1N045_BOVIN]	Successfully Matured: In Vivo	2	2	1.89	4074.15	5253.25	1596.70	221.56	34.70	8.51	1.00	2.40	3.35	0.0044	InVivo/SM>InVivo/FM
tr|F1N6P2|F1N6P2_BOVIN	Uncharacterized protein OS=Bos taurus GN=SURF1 PE=4 SV=2	Successfully Matured: In Vivo	9	9	8.94	151164.85	40039.18	64333.65	26341.10	2.75	12.59	0.29	11.50	0.55	0.0045	InVivo/SM>InVivo/FM
sp|Q3T0N3|TMCO1_BOVIN	Transmembrane and coiled-coil domain-containing protein 1 OS=Bos taurus GN=TMCO1 PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.91	17458.00	3555.35	7468.91	3769.74	2.60	10.44	0.20	9.37	0.58	0.0045	InVivo/SM>InVivo/FM
sp|Q28085|CFAH_BOVIN	Complement factor H OS=Bos taurus GN=CFH PE=1 SV=3	Successfully Matured: In Vivo	7	5	6.84	43101.53	37769.71	15890.84	1001.43	39.32	10.85	1.29	6.91	1.85	0.0045	InVivo/SM>InVivo/FM; InVitro/FM>InVivo/FM
tr|K4JF16|K4JF16_BOVIN	Alpha-2-macroglobulin variant 23 OS=Bos taurus GN=A2M PE=2 SV=1	Successfully Matured: In Vivo	12	1	11.83	12282.79	17733.17	4814.81	0.00	Infinity	7.84	4.50	0.00	0.00	0.0046	InVivo/FM>InVitro/FM
sp|Q3MHX6|OS9_BOVIN	Protein OS-9 OS=Bos taurus GN=OS9 PE=2 SV=1 [sp|Q3MHX6|OS9_BOVIN,tr|F1M3651|F1M365_BOVIN]	Successfully Matured: In Vivo	3	3	2.98	41142.68	27847.93	16916.18	3815.76	4.41	11.13	0.67	9.76	0.43	0.0048	InVivo/SM>InVivo/FM
sp|A6QLY7|PBIP1_BOVIN	Pre-B-cell leukemia transcription factor-interacting protein 1 OS=Bos taurus GN=PBXIP1 PE=2 SV=1	Successfully Matured: In Vivo	9	9	8.95	99464.84	60830.52	41686.86	7046.07	3.70	12.04	0.63	10.86	0.28	0.0048	
tr|E1BMM8|E1BMM8_BOVIN	Uncharacterized protein OS=Bos taurus GN=CHPF PE=4 SV=1	Successfully Matured: In Vivo	5	5	4.97	20342.91	14897.56	7774.72	2834.44	9.50	10.38	0.78	7.54	1.45	0.0049	InVivo/SM>InVivo/FM
sp|Q3T067|SCPDL_BOVIN	Saccharopine dehydrogenase-like oxidoreductase OS=Bos taurus GN=SCCPDH PE=2 SV=1 [sp|Q3T067|SCPDL_BOVIN,tr|F1MX05|F1MX05_BOVIN]	Successfully Matured: In Vivo	5	5	4.96	265857.33	114450.73	117518.79	53017.73	2.54	13.12	0.36	12.17	0.42	0.0050	
tr|F1MYG0|F1MYG0_BOVIN	Ornithine aminotransferase, mitochondrial OS=Bos taurus GN=OAT PE=3 SV=1	Successfully Matured: In Vivo	47	47	46.44	10684822.71	5810641.04	4326632.04	2003514.51	4.35	16.76	0.54	15.17	0.75	0.0050	InVivo/SM>InVivo/FM
sp|Q8SPP7|PGRP1_BOVIN	Peptidoglycan recognition protein 1 OS=Bos taurus GN=PGLYRP1 PE=1 SV=1 [sp|Q8SPP7|PGRP1_BOVIN,tr|H2CNR1|H2CNR1_BOVIN]	Successfully Matured: In Vivo	3	3	2.94	29756.45	23788.23	10840.24	485.54	41.63	10.33	1.74	6.98	0.92	0.0052	InVivo/SM>InVivo/FM
sp|Q28042|OVGP1_BOVIN	Oviduct-specific glycoprotein (Fragment) OS=Bos taurus GN=OVGP1 PE=1 SV=1 [sp|Q28042|OVGP1_BOVIN,tr|A1L579|A1L579_BOVIN]	Successfully Matured: In Vivo	2	2	1.91	11127.47	16124.89	4400.88	286.22	82.84	8.86	2.21	2.61	3.00	0.0056	InVivo/SM>InVivo/FM
tr|E1BHQ9|E1BHQ9_BOVIN	Uncharacterized protein OS=Bos taurus GN=MCAM PE=4 SV=1	Successfully Matured: In Vivo	14	14	13.80	195410.26	119182.72	77479.37	43968.24	5.42	12.74	0.58	10.68	1.08	0.0056	
tr|F1MKS5|F1MKS5_BOVIN	Histidine-rich glycoprotein OS=Bos taurus GN=HRG PE=4 SV=2 [tr|F1MKS5|F1MKS5_BOVIN,tr|Q9BGU1|Q9BGU1_BOVIN]	Successfully Matured: In Vivo	3	1	2.95	370.76	473.73	143.77	0.00	Infinity	5.05	3.02	0.00	0.00	0.0057	InVivo/SM>InVivo/FM
sp|P80747|ITB5_BOVIN	Integrin beta-5 OS=Bos taurus GN=ITGB5 PE=1 SV=2	Successfully Matured: In Vivo	7	7	6.88	109286.43	123851.08	41953.06	3259.22	31.28	11.51	1.55	8.51	0.90	0.0058	InVivo/SM>InVivo/FM
sp|Q05B71|CISD2_BOVIN	CDGSH iron-sulfur domain-containing protein 2 OS=Bos taurus GN=CISD2 PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.89	102394.97	29750.70	45407.45	22275.62	2.34	12.20	0.28	11.29	0.47	0.0059	
tr|A6QNS6|A6QNS6_BOVIN	NID1 protein OS=Bos taurus GN=NID1 PE=2 SV=1 [tr|A6QNS6|A6QNS6_BOVIN,tr|F1MWN3|F1MWN3_BOVIN]	Successfully Matured: In Vivo	8	8	7.96	60245.64	71385.33	23270.11	2012.85	32.72	10.87	1.53	7.74	1.10	0.0059	InVivo/SM>InVivo/FM
sp|P18246|CXA1_BOVIN	Gap junction alpha-1 protein OS=Bos taurus GN=GJA1 PE=2 SV=2	Successfully Matured: In Vivo	21	21	20.63	2671723.16	1233809.60	1137706.28	534619.14	3.07	15.42	0.40	14.22	0.60	0.0061	InVivo/SM>InVivo/FM
sp|P07994|INHA_BOVIN	Inhibin alpha chain OS=Bos taurus GN=INHA PE=1 SV=1	Successfully Matured: In Vivo	12	12	11.67	298256.65	187330.25	113817.11	36174.50	8.04	13.10	0.76	10.70	1.26	0.0064	
sp|Q3MHG1|SPTC1_BOVIN	Serine palmitoyltransferase 1 OS=Bos taurus GN=SPTLC1 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.99	24417.31	11027.84	9678.10	9539.50	4.64	10.71	0.45	8.14	1.51	0.0064	InVivo/SM>InVivo/FM
sp|Q2TBX5|SSRD_BOVIN	Translocon-associated protein subunit delta OS=Bos taurus GN=SSR4 PE=2 SV=1	Successfully Matured: In Vivo	6	6	5.98	258931.48	50309.53	117310.58	53413.41	2.07	13.14	0.21	12.36	0.43	0.0064	InVivo/SM>InVivo/FM
tr|G3MZJ1|G3MZJ1_BOVIN	Uncharacterized protein OS=Bos taurus GN=LOC101903062 PE=4 SV=1	Successfully Matured: In Vivo	2	2	1.99	74813.91	40242.65	29108.38	18623.54	5.86	11.81	0.49	9.31	1.46	0.0067	
sp|P23805|CONG_BOVIN;sp|P62916|CL43_BOVIN;tr|B7FEK7|B7FEK7_BOVIN;tr|F1MFY6|F1MFY6_BOVIN	Conglutinin OS=Bos taurus GN=CGN1 PE=1 SV=2	Successfully Matured: In Vivo	10	10	9.81	309791.36	198881.93	109636.48	2407.56	61.02	12.61	2.06	9.06	0.74	0.0067	InVivo/SM>InVivo/FM
tr|E1BF27|E1BF27_BOVIN	Uncharacterized protein OS=Bos taurus GN=SLC38A10 PE=4 SV=2	Successfully Matured: In Vivo	2	2	1.80	17929.82	13542.31	6732.05	1979.83	13.33	10.26	0.76	6.54	2.17	0.0068	
sp|O18824|SCRB1_BOVIN	Scavenger receptor class B member 1 OS=Bos taurus GN=SCARB1 PE=2 SV=1 [sp|O18824|SCRB1_BOVIN,tr|A4IFC6|A4IFC6_BOVIN]	Successfully Matured: In Vivo	2	2	1.94	67982.05	50875.11	26773.81	6952.20	6.82	11.56	0.86	9.67	0.79	0.0068	InVivo/SM>InVivo/FM
sp|Q4PJW3|CP51A_BOVIN	Lanosterol 14-alpha demethylase OS=Bos taurus GN=CYP51A1 PE=2 SV=1	Successfully Matured: In Vivo	5	5	4.95	35173.32	21977.27	14581.67	5019.17	4.00	11.00	0.65	9.66	0.51	0.0069	InVivo/SM>InVivo/FM
sp|P15497|APOA1_BOVIN	Apolipoprotein A-I OS=Bos taurus GN=APOA1 PE=1 SV=3 [sp|P15497|APOA1_BOVIN,tr|V6F9A2|V6F9A2_BOVIN]	Successfully Matured: In Vivo	5	5	4.94	141430.32	69266.53	52669.35	22163.66	8.54	12.48	0.41	9.29	1.95	0.0072	InVivo/SM>InVivo/FM
sp|P02769|ALBU_BOVIN;zz|ZZ_FGCZCont0112|	sp|ALBU_BOVIN| [sp|P02769|ALBU_BOVIN,zz|ZZ_FGCZCont0112|]	Successfully Matured: In Vivo	290	13	284.93	21310501.54	13657548.02	8292591.51	3134993.34	6.64	17.35	0.81	15.27	1.03	0.0073	InVivo/SM>InVivo/FM
sp|Q5E980|CP20A_BOVIN	Cytochrome P450 20A1 OS=Bos taurus GN=CYP20A1 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.98	1344.04	2011.13	587.27	464.19	6.47	7.25	1.12	1.57	3.40	0.0074	
tr|Q0P5H6|Q0P5H6_BOVIN	Transducin (Beta)-like 2 OS=Bos taurus GN=TBL2 PE=2 SV=1	Successfully Matured: In Vivo	6	5	5.79	66898.34	10284.81	30091.50	15288.26	2.09	11.79	0.17	10.97	0.49	0.0075	
tr|Q5E9F1|Q5E9F1_BOVIN	B-cell receptor-associated protein 31 OS=Bos taurus GN=BCAP31 PE=2 SV=1	Successfully Matured: In Vivo	7	7	6.87	507821.82	201801.48	234534.30	65587.36	2.10	13.78	0.36	13.06	0.28	0.0075	
tr|F1N672|F1N672_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=TM9SF2 PE=4 SV=2	Successfully Matured: In Vivo	11	11	10.72	398773.18	209787.34	159893.39	105798.75	4.58	13.45	0.62	11.63	0.96	0.0076	InVivo/SM>InVivo/FM
tr|A5PJD6|A5PJD6_BOVIN	ATL3 protein OS=Bos taurus GN=ATL3 PE=2 SV=1	Successfully Matured: In Vivo	9	9	8.59	99436.20	60154.85	40514.88	14883.58	4.40	12.01	0.72	10.58	0.54	0.0077	InVivo/SM>InVivo/FM

						Successfully Matured: In Vivo		Successfully Matured: In Vitro			Successfully Matured: In Vivo		Successfully Matured: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|A1L539|A1L539_BOVIN	Glypican 6 OS=Bos taurus GN=GPC6 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.99	16687.94	13625.52	6373.05	1044.03	11.47	9.97	1.20	7.78	0.68	0.0077	InVivo/SM>InVivo/FM
sp|P32007|ADT3_BOVIN;sp|Q2YDD9|ADT4_BOVIN;tr|F1MDK8|F1MDK8_BOVIN	ADP/ATP translocase 3 OS=Bos taurus GN=SLC25A6 PE=1 SV=3	Successfully Matured: In Vivo	26	7	25.19	806776.48	333205.04	364596.39	113189.17	2.30	14.23	0.40	13.42	0.32	0.0079	InVivo/SM>InVivo/FM
sp|A0JNK3|HTRA2_BOVIN	Serine protease HTRA2, mitochondrial OS=Bos taurus GN=HTRA2 PE=2 SV=1	Successfully Matured: In Vivo	5	5	4.97	27269.00	12167.93	11719.42	5502.29	2.91	10.82	0.47	9.72	0.53	0.0081	
sp|A2VE53|IKIP_BOVIN	Inhibitor of nuclear factor kappa-B kinase-interacting protein OS=Bos taurus GN=IKBIP PE=2 SV=1	Successfully Matured: In Vivo	9	9	8.71	311039.47	74431.25	142444.84	67366.06	2.03	13.32	0.24	12.56	0.43	0.0084	
sp|Q1LZH1|MARC2_BOVIN	Mitochondrial amidoxime reducing component 2 OS=Bos taurus GN=MARC2 PE=2 SV=1 [sp|Q1LZH1|MARC2_BOVIN;tr|G3X6L2|G3X6L2_BOVIN]	Successfully Matured: In Vivo	2	2	1.99	8229.20	5848.05	3218.66	1014.06	7.00	9.40	1.00	7.55	0.66	0.0085	
tr|A6QNM9|A6QNM9_BOVIN	SLC25A12 protein OS=Bos taurus GN=SLC25A12 PE=2 SV=1	Successfully Matured: In Vivo	7	5	6.92	42966.88	26044.44	18119.89	6929.97	3.56	11.22	0.58	9.96	0.57	0.0086	InVivo/SM>InVivo/FM
tr|F1MRT9|F1MRT9_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=UTRN PE=4 SV=2	Successfully Matured: In Vivo	5	5	4.87	22971.26	12277.35	9736.21	3513.15	3.30	10.62	0.54	9.43	0.56	0.0087	InVivo/FM>InVitro/FM
tr|Q2KJ71|Q2KJ71_BOVIN	Transformer 2 alpha homolog (Drosophila) OS=Bos taurus GN=TRA2A PE=2 SV=1	Successfully Matured: In Vivo	4	3	3.86	66576.26	17510.52	28215.90	25213.51	2.80	11.77	0.28	10.45	0.82	0.0090	
sp|Q2KHX3|PRAF2_BOVIN	PRA1 family protein 2 OS=Bos taurus GN=PRAF2 PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.91	211837.35	46269.07	97100.47	42429.58	2.01	12.94	0.20	12.19	0.45	0.0090	
tr|E1B828|E1B828_BOVIN	Uncharacterized protein OS=Bos taurus GN=LEMD2 PE=4 SV=1	Successfully Matured: In Vivo	4	4	3.98	41954.30	12681.66	18378.46	8972.51	2.48	11.30	0.29	10.31	0.58	0.0090	
tr|G3MZ03|G3MZ03_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=A4GALT PE=4 SV=1	Successfully Matured: In Vivo	2	2	1.96	3393.52	2961.73	1251.19	120.04	42.45	8.04	1.79	2.56	3.10	0.0090	InVivo/SM>InVivo/FM
tr|E1B6Z6|E1B6Z6_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=LCN2 PE=3 SV=2	Successfully Matured: In Vivo	2	2	1.97	4291.69	4459.46	1605.83	67.08	85.60	8.09	2.02	2.89	2.74	0.0091	InVivo/SM>InVivo/FM
tr|Q2KIC3|Q2KIC3_BOVIN	HTATSF1 protein (Fragment) OS=Bos taurus GN=HTATSF1 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.95	14565.24	6721.26	5551.62	2657.75	6.44	10.16	0.60	7.76	1.46	0.0094	
sp|Q0VCG9|PTX3_BOVIN	Pentraxin-related protein PTX3 OS=Bos taurus GN=PTX3 PE=2 SV=1	Successfully Matured: In Vivo	11	11	10.81	475571.23	558541.69	185169.86	5738.95	22.39	12.95	1.51	10.63	0.28	0.0095	InVivo/SM>InVivo/FM
tr|F1MJ36|F1MJ36_BOVIN	Uncharacterized protein OS=Bos taurus GN=LOC539818 PE=4 SV=1	Successfully Matured: In Vivo	7	1	6.81	21026.34	10108.79	8816.63	2299.56	3.37	10.51	0.65	9.38	0.36	0.0095	
sp|A5PJ65|RER1_BOVIN	Protein RER1 OS=Bos taurus GN=RER1 PE=2 SV=2	Successfully Matured: In Vivo	4	4	3.98	104741.84	33017.86	44695.61	23300.42	2.79	12.21	0.33	11.05	0.70	0.0095	
sp|Q0VCY0|AT2A1_BOVIN	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 OS=Bos taurus GN=ATP2A1 PE=1 SV=1 [sp|Q0VCY0|AT2A1_BOVIN;tr|F1MGE7|F1MGE7_BOVIN]	Successfully Matured: In Vivo	8	2	7.81	119052.04	58518.58	51928.44	24368.32	2.79	12.28	0.50	11.26	0.46	0.0097	InVivo/SM>InVivo/FM
sp|Q2KID7|OSTC_BOVIN	Oligosaccharyltransferase complex subunit OSTC OS=Bos taurus GN=OSTC PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.90	167375.10	72831.65	73823.28	40084.43	2.57	12.65	0.42	11.66	0.51	0.0099	
sp|Q5ICW4|GRB14_BOVIN	Growth factor receptor-bound protein 14 OS=Bos taurus GN=GRB14 PE=1 SV=1 [sp|Q5ICW4|GRB14_BOVIN;tr|F1MBA2|F1MBA2_BOVIN]	Successfully Matured: In Vivo	4	4	3.86	11855.40	1008.68	4991.01	3982.75	2.62	10.07	0.08	8.82	0.83	0.0099	
sp|P53793|SC5A3_BOVIN	Sodium/myo-inositol cotransporter OS=Bos taurus GN=SLC5A3 PE=3 SV=1 [sp|P53793|SC5A3_BOVIN;tr|O19135|O19135_BOVIN]	Successfully Matured: In Vivo	2	2	1.99	20704.52	4575.24	9123.83	4931.19	2.33	10.61	0.25	9.66	0.58	0.0100	
tr|Q3ZCA7|Q3ZCA7_BOVIN	Guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3 OS=Bos taurus GN=GNAI3 PE=2 SV=1	Successfully Matured: In Vivo	7	3	6.82	51959.67	13496.81	23849.72	11423.12	2.03	11.53	0.25	10.76	0.44	0.0100	
sp|Q3SZ45|SDF2_BOVIN	Stromal cell-derived factor 2 OS=Bos taurus GN=SDF2 PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.88	20725.46	20350.48	8216.31	3743.96	9.13	10.18	1.16	7.56	1.32	0.0102	
tr|G3MWT1|G3MWT1_BOVIN	Uncharacterized protein OS=Bos taurus GN=LOC101905477 PE=4 SV=1 [tr|G3MWT1|G3MWT1_BOVIN;tr|G3N148|G3N148_BOVIN;tr|G3N1H5|G3N1H5_BOVIN;tr|G3N1R1|G3N1R1_BOVIN]	Successfully Matured: In Vivo	2	2	1.99	35403.76	34631.02	13490.65	2319.58	19.23	10.48	1.55	7.73	0.99	0.0103	InVivo/SM>InVivo/FM
sp|Q8D863-2|RTN3_BOVIN	Isoform 2 of Reticulon-3 OS=Bos taurus GN=RTN3	Successfully Matured: In Vivo	3	3	2.90	61834.16	19403.67	25521.49	19279.98	3.27	11.68	0.33	10.15	0.97	0.0103	
tr|F1MVK1|F1MVK1_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=2	Successfully Matured: In Vivo	11	1	10.89	7692.51	6207.83	2840.31	319.55	24.86	9.00	1.64	5.90	1.27	0.0103	InVivo/SM>InVivo/FM
sp|Q3ZKN0|S27A1_BOVIN	Long-chain fatty acid transport protein 1 OS=Bos taurus GN=SLC27A1 PE=2 SV=1 [sp|Q3ZKN0|S27A1_BOVIN;tr|A4IFM2|A4IFM2_BOVIN]	Successfully Matured: In Vivo	7	7	6.94	78459.72	33638.61	31905.69	15796.63	3.86	11.89	0.45	10.31	0.96	0.0105	
tr|A5PKH0|A5PKH0_BOVIN	TOR1AIP2 protein OS=Bos taurus GN=TOR1AIP2 PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.96	49726.47	27090.99	20929.85	8788.90	3.45	11.38	0.57	10.11	0.64	0.0106	InVivo/SM>InVivo/FM
tr|E1BJ75|E1BJ75_BOVIN	Uncharacterized protein OS=Bos taurus GN=CRTAP PE=4 SV=1	Successfully Matured: In Vivo	8	8	7.84	72197.36	36458.47	31714.17	16923.69	2.72	11.79	0.48	10.76	0.49	0.0106	
sp|Q0VCM5|ITIH1_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Bos taurus GN=ITIH1 PE=2 SV=1	Successfully Matured: In Vivo	88	7	87.15	695937.29	826212.56	264901.80	2642.33	111.46	12.94	2.38	9.35	0.46	0.0106	InVivo/SM>InVivo/FM
sp|P21398|AOFA_BOVIN	Amine oxidase [flavin-containing] A OS=Bos taurus GN=MAOA PE=2 SV=2	Successfully Matured: In Vivo	13	13	12.66	549854.78	332042.74	240641.54	55122.13	2.92	13.77	0.59	12.80	0.29	0.0110	InVivo/SM>InVivo/FM
sp|Q3ZBA6|DJB11_BOVIN	DnaJ homolog subfamily B member 11 OS=Bos taurus GN=DNAJB11 PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.99	125000.84	65525.85	53068.28	29599.37	3.24	12.31	0.55	11.07	0.64	0.0111	InVivo/SM>InVivo/FM
sp|P00171|CYB5_BOVIN	Cytochrome b5 OS=Bos taurus GN=CYB5A PE=1 SV=3	Successfully Matured: In Vivo	7	3	6.96	197237.52	165582.02	79076.44	39769.21	6.43	12.56	0.96	10.54	0.98	0.0111	
sp|Q3T134|SPCS1_BOVIN	Signal peptidase complex subunit 1 OS=Bos taurus GN=SPCS1 PE=3 SV=1	Successfully Matured: In Vivo	2	2	1.96	88473.09	54969.23	35838.67	27628.18	4.65	11.93	0.63	9.81	1.30	0.0114	
tr|E1BH79|E1BH79_BOVIN	Uncharacterized protein OS=Bos taurus GN=EFTUD1 PE=4 SV=2	Successfully Matured: In Vivo	4	4	3.82	8672.95	4331.09	3663.30	2904.26	3.29	9.65	0.54	8.23	0.82	0.0120	InVivo/SM>InVivo/FM
tr|Q2HJ65|Q2HJ65_BOVIN	Histone H2A OS=Bos taurus GN=H2AFY PE=2 SV=1	Successfully Matured: In Vivo	11	10	10.71	654489.13	292241.79	301427.59	121067.69	2.17	14.02	0.40	13.26	0.34	0.0122	
sp|A5D7L5|S39AE_BOVIN	Zinc transporter ZIP14 OS=Bos taurus GN=SLC39A14 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.99	32410.83	15593.59	12820.44	11590.50	4.84	10.97	0.56	8.13	1.89	0.0123	InVivo/FM>InVitro/FM
tr|A6QR21|A6QR21_BOVIN	Mitochondrial carrier homolog 2 OS=Bos taurus GN=MTCH2 PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.87	104688.44	56948.67	45730.12	19297.85	2.85	12.15	0.50	11.09	0.53	0.0124	
tr|Q0VCQ9|Q0VCQ9_BOVIN	Reticulocalbin 2, EF-hand calcium binding domain OS=Bos taurus GN=RCN2 PE=2 SV=1	Successfully Matured: In Vivo	9	9	8.88	233278.49	159924.01	93562.41	45232.68	5.35	12.82	0.83	10.98	0.98	0.0127	InVivo/SM>InVivo/FM
tr|A5D7D5|A5D7D5_BOVIN	MATN2 protein OS=Bos taurus GN=MATN2 PE=2 SV=1 [tr|A5D7D5|A5D7D5_BOVIN;tr|F1MHR8|F1MHR8_BOVIN]	Successfully Matured: In Vivo	2	2	1.94	1344.03	1925.71	602.98	636.35	4.72	7.07	1.44	1.59	3.56	0.0127	
sp|Q3SZ87|SSRG_BOVIN	Translocon-associated protein subunit gamma OS=Bos taurus GN=SSR3 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.94	121237.71	68226.12	52507.61	33088.35	3.00	12.29	0.52	11.10	0.66	0.0131	InVivo/SM>InVivo/FM
sp|O77588|PLOD1_BOVIN	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 OS=Bos taurus GN=PLOD1 PE=2 SV=2	Successfully Matured: In Vivo	35	33	34.28	2255737.08	785881.69	984811.80	608953.69	2.57	15.28	0.33	14.18	0.69	0.0132	InVivo/FM>InVitro/FM
tr|G8JKY2|G8JKY2_BOVIN	7-dehydrocholesterol reductase OS=Bos taurus GN=DHCR7 PE=4 SV=1	Successfully Matured: In Vivo	9	9	8.82	263203.58	143050.61	108783.85	71025.48	3.82	13.06	0.55	11.42	1.02	0.0132	InVivo/SM>InVivo/FM
tr|G3MX98|G3MX98_BOVIN	Uncharacterized protein OS=Bos taurus GN=KIF9 PE=4 SV=1	Successfully Matured: In Vivo	3	1	2.79	247666.82	201607.92	97716.27	28297.76	7.33	12.77	1.00	10.77	1.00	0.0132	InVivo/SM>InVivo/FM; InVitro/FM>InVivo/FM
sp|Q3ZC50|CCD47_BOVIN	Coiled-coil domain-containing protein 47 OS=Bos taurus GN=CCDC47 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.99	43072.74	27401.87	17814.52	15154.72	4.10	11.18	0.70	9.35	1.10	0.0135	
tr|A4IF88|A4IF88_BOVIN	RCN1 protein OS=Bos taurus GN=RCN1 PE=2 SV=1	Successfully Matured: In Vivo	6	6	5.95	312551.49	108953.39	144322.86	62724.45	2.06	13.30	0.34	12.55	0.41	0.0141	
sp|Q2NKR7|F162A_BOVIN	Protein FAM162A OS=Bos taurus GN=FAM162A PE=2 SV=1 [sp|Q2NKR7|F162A_BOVIN;tr|F1N3S2|F1N3S2_BOVIN]	Successfully Matured: In Vivo	4	4	3.99	261462.14	107924.72	105743.95	60074.73	3.93	13.09	0.45	11.34	1.18	0.0143	
tr|Q3ZBB0|Q3ZBB0_BOVIN	Solute carrier family 35, member B2 OS=Bos taurus GN=SLC35B2 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.84	31245.88	9764.92	12969.61	7059.00	3.18	11.01	0.29	9.56	1.00	0.0144	
sp|P05632|ATP5E_BOVIN	ATP synthase subunit epsilon, mitochondrial OS=Bos taurus GN=ATP5E PE=1 SV=4	Successfully Matured: In Vivo	2	2	1.89	24597.47	8379.61	11139.43	4583.24	2.21	10.76	0.32	9.92	0.52	0.0144	
sp|A5D7E2|TM9S4_BOVIN	Transmembrane 9 superfamily member 4 OS=Bos taurus GN=TM9SF4 PE=2 SV=2	Successfully Matured: In Vivo	5	5	4.98	74474.79	38668.24	31518.93	22653.84	3.29	11.80	0.55	10.40	0.85	0.0148	
sp|P41563|IDH3A_BOVIN	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial OS=Bos taurus GN=IDH3A PE=1 SV=1 [sp|P41563|IDH3A_BOVIN;tr|F1MN74|F1MN74_BOVIN;tr|Q148J8|Q148J8_BOVIN]	Successfully Matured: In Vivo	15	15	14.87	718655.31	645222.21	293156.12	77125.71	5.86	13.82	0.95	12.24	0.66	0.0154	

						Successfully Matured: In Vivo		Successfully Matured: In Vitro			Successfully Matured: In Vivo		Successfully Matured: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
sp|Q3SZB7|F16P1_BOVIN	Fructose-1,6-bisphosphatase 1 OS=Bos taurus GN=FBP1 PE=2 SV=3	Successfully Matured: In Vivo	3	3	2.97	17865.05	18208.14	7217.69	3671.06	7.65	10.00	1.12	7.66	1.28	0.0156	InVivo/SM>InVivo/FM
sp|P80513|MANF_BOVIN	Mesencephalic astrocyte-derived neurotrophic factor OS=Bos taurus GN=MANF PE=1 SV=2	Successfully Matured: In Vivo	14	14	13.66	1425026.00	612540.71	650569.29	243452.53	2.23	14.79	0.43	14.00	0.38	0.0158	
tr|Q1IH5|Q1IH5_BOVIN	Nucleobindin 2 OS=Bos taurus GN=NUCB2 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.99	16214.27	15638.84	6605.44	4280.63	6.48	10.01	1.03	7.45	1.57	0.0159	
tr|Q0P5F6|Q0P5F6_BOVIN	CYB5B protein OS=Bos taurus GN=CYB5B PE=2 SV=2	Successfully Matured: In Vivo	6	6	5.96	305053.46	186668.77	128840.62	47071.72	3.54	13.14	0.70	11.94	0.54	0.0162	
sp|Q2KJ39|RCN3_BOVIN	Reticulocalbin-3 OS=Bos taurus GN=RCN3 PE=2 SV=1	Successfully Matured: In Vivo	16	15	15.62	711184.77	279457.96	331360.32	170230.84	2.03	14.11	0.35	13.38	0.41	0.0163	
sp|Q27966-2|MYO1C_BOVIN	Isoform 2 of Unconventional myosin-Ic OS=Bos taurus GN=MYO1C [sp|Q27966-2|MYO1C_BOVIN,sp|Q27966-3|MYO1C_BOVIN,sp|Q27966|MYO1C_BOVIN]	Successfully Matured: In Vivo	2	2	1.91	1657206.40	1255945.43	649226.83	234778.92	7.29	14.69	1.04	12.48	1.26	0.0165	InVivo/SM>InVivo/FM; InVitro/FM>InVivo/FM
sp|P00257-2|ADX_BOVIN	Isoform 2 of Adrenodoxin, mitochondrial OS=Bos taurus GN=FDX1 [sp|P00257-2|ADX_BOVIN,sp|P00257|ADX_BOVIN,tr|F1N3J0|F1N3J0_BOVIN]	Successfully Matured: In Vivo	3	3	2.99	186253.95	170845.61	76467.93	12784.14	5.66	12.45	0.97	11.03	0.41	0.0166	InVivo/SM>InVivo/FM
sp|P84466|ERG7_BOVIN	Lanosterol synthase OS=Bos taurus GN=LSS PE=1 SV=2 [sp|P84466|ERG7_BOVIN,tr|F1MT99|F1MT99_BOVIN]	Successfully Matured: In Vivo	19	19	18.59	601936.03	241742.34	276781.08	80301.26	2.14	13.93	0.45	13.21	0.30	0.0169	
sp|P00743|FA10_BOVIN	Coagulation factor X OS=Bos taurus GN=F10 PE=1 SV=1 [sp|P00743|FA10_BOVIN,tr|Q3MHW2|Q3MHW2_BOVIN]	Successfully Matured: In Vivo	7	7	6.95	78191.27	97095.31	30754.53	4536.51	21.69	11.01	1.63	8.10	1.44	0.0174	InVivo/SM>InVivo/FM
tr|F1MTY9|F1MTY9_BOVIN	Heme oxygenase (Decycling) 2 OS=Bos taurus GN=HMOX2 PE=4 SV=2	Successfully Matured: In Vivo	7	7	6.89	231333.83	138268.64	104430.24	24985.83	2.53	12.91	0.56	12.09	0.26	0.0177	InVivo/SM>InVivo/FM
sp|Q1LZ95-2|IDI1_BOVIN	Isoform 2 of Isopentenyl-diphosphate Delta-isomerase 1 OS=Bos taurus GN=IDI1 [sp|Q1LZ95-2|IDI1_BOVIN,sp|Q1LZ95|IDI1_BOVIN]	Successfully Matured: In Vivo	4	4	3.96	16786.45	16290.21	6961.35	4843.44	5.52	10.12	0.82	7.42	1.86	0.0177	
tr|E1BBT8|E1BBT8_BOVIN	Uncharacterized protein OS=Bos taurus GN=EMC1 PE=4 SV=2	Successfully Matured: In Vivo	4	4	3.93	78908.46	20103.19	35485.43	16719.85	2.18	11.94	0.26	11.06	0.62	0.0187	InVivo/SM>InVivo/FM
sp|Q3ZBQ0|SIR5_BOVIN	NAD-dependent protein deacylase sirtuin-5, mitochondrial OS=Bos taurus GN=SIRT5 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.93	25741.70	13634.05	10750.38	4391.68	3.56	10.69	0.70	9.41	0.69	0.0196	
tr|B0JYQ0|B0JYQ0_BOVIN	ALB protein OS=Bos taurus GN=ALB PE=2 SV=1	Successfully Matured: In Vivo	265	24	260.40	1793789.59	1202116.27	706015.79	241924.21	6.19	14.82	0.92	12.78	1.28	0.0197	InVivo/SM>InVivo/FM
tr|F6RM11|F6RM11_BOVIN	Uncharacterized protein OS=Bos taurus GN=NPTN PE=4 SV=1	Successfully Matured: In Vivo	3	3	2.96	107622.59	63970.49	46503.64	38321.35	3.10	12.13	0.62	10.82	0.80	0.0199	InVivo/SM>InVivo/FM
tr|A5D9A8|A5D9A8_BOVIN	Squalene epoxidase OS=Bos taurus GN=SQLE PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.99	9363.02	4598.95	3837.51	3260.62	3.88	9.68	0.70	7.77	1.30	0.0199	
tr|Q6QRN7|Q6QRN7_BOVIN	PP1201 protein OS=Bos taurus GN=REC51 PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.95	31009.38	18154.07	13207.46	4726.04	3.32	10.93	0.49	9.62	0.88	0.0200	
sp|Q5EA53|T2FA_BOVIN	General transcription factor IIF subunit 1 OS=Bos taurus GN=GTF2F1 PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.99	48317.08	14212.38	20851.38	23483.08	2.63	11.44	0.32	9.99	1.07	0.0205	
tr|F1ML49|F1ML49_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=KIAA0319L PE=4 SV=1	Successfully Matured: In Vivo	2	2	1.97	11088.06	6004.13	4685.42	1986.55	3.37	9.88	0.58	8.57	0.83	0.0209	
sp|Q3ZCD7|TECR_BOVIN	Very-long-chain enoyl-CoA reductase OS=Bos taurus GN=TECR PE=2 SV=1	Successfully Matured: In Vivo	5	5	4.89	114463.22	47300.52	52664.52	25004.49	2.15	12.27	0.41	11.49	0.45	0.0211	
tr|Q17QH1|Q17QH1_BOVIN	APOF protein OS=Bos taurus GN=APOF PE=2 SV=2	Successfully Matured: In Vivo	2	2	1.98	5787.48	11999.40	2472.72	27.09	137.24	7.40	2.09	3.77	1.92	0.0212	
tr|E1B7B1|E1B7B1_BOVIN	Uncharacterized protein OS=Bos taurus GN=SEC63 PE=4 SV=1	Successfully Matured: In Vivo	6	6	5.93	53420.27	32723.58	22677.65	15971.34	3.44	11.45	0.56	9.86	1.11	0.0212	
tr|F1MT42|F1MT42_BOVIN	Follicle-stimulating hormone receptor OS=Bos taurus GN=FSHR PE=3 SV=2	Successfully Matured: In Vivo	2	2	1.98	9273.05	2457.91	3830.56	2282.33	3.17	9.80	0.28	8.24	1.19	0.0213	InVivo/FM>InVivo/SM; InVivo/FM>InVitro/FM
sp|Q0BDB5|STX5_BOVIN	Syntaxin-5 OS=Bos taurus GN=STX5 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.99	33875.42	14299.16	14839.75	12924.78	2.63	11.05	0.45	9.84	0.83	0.0213	
sp|P03972|MIS_BOVIN	Muellerian-inhibiting factor OS=Bos taurus GN=AMH PE=1 SV=1	Successfully Matured: In Vivo	5	5	4.94	17144.55	8222.45	6832.97	6793.92	4.59	10.32	0.59	7.26	2.34	0.0218	
sp|A1A4P9|CC167_BOVIN	Coiled-coil domain-containing protein 167 OS=Bos taurus GN=CCDC167 PE=4 SV=1 [sp|A1A4P9|CC167_BOVIN,tr|G3MWY8|G3MWY8_BOVIN,tr|G3N3U2|G3N3U2_BOVIN]	Successfully Matured: In Vivo	2	2	1.84	8554.11	2004.25	3453.25	2237.22	3.50	9.72	0.28	7.95	1.37	0.0220	
tr|A6QLZ3|A6QLZ3_BOVIN	ARSB protein OS=Bos taurus GN=ARSB PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.89	18000.76	13731.32	7580.52	4514.03	4.05	10.27	0.74	8.70	1.00	0.0224	
sp|Q28035|GSTA1_BOVIN	Glutathione S-transferase A1 OS=Bos taurus GN=GSTA1 PE=2 SV=3	Successfully Matured: In Vivo	57	29	56.42	34804369.52	14044224.35	15685350.61	6268587.16	2.32	17.98	0.48	17.14	0.46	0.0225	
sp|Q5EA40|BCAT2_BOVIN	Branched-chain-amino-acid aminotransferase, mitochondrial OS=Bos taurus GN=BCAT2 PE=2 SV=1 [sp|Q5EA40|BCAT2_BOVIN,tr|Q0V8J6|Q0V8J6_BOVIN]	Successfully Matured: In Vivo	4	4	3.95	251817.43	293710.92	99305.86	24895.60	15.33	12.25	1.62	9.57	1.38	0.0229	InVivo/SM>InVivo/FM
tr|F1MIU7|F1MIU7_BOVIN	Uncharacterized protein OS=Bos taurus GN=SLC27A3 PE=4 SV=2	Successfully Matured: In Vivo	4	4	3.93	13890.38	14767.13	5282.33	369.70	31.61	9.27	1.89	6.21	1.54	0.0231	
tr|E1BB91|E1BB91_BOVIN	Uncharacterized protein OS=Bos taurus GN=COL6A3 PE=4 SV=1	Successfully Matured: In Vivo	2	2	1.88	1512.31	2090.11	594.74	42.96	78.72	6.13	3.30	1.05	2.35	0.0231	InVivo/SM>InVivo/FM
sp|Q5SYT6|CLGN_BOVIN	Calmegin OS=Bos taurus GN=CLGN PE=2 SV=1 [sp|Q5SYT6|CLGN_BOVIN,tr|Q2EGT5|Q2EGT5_BOVIN]	Successfully Matured: In Vivo	5	4	4.96	21577.54	25076.26	8309.54	688.63	32.89	9.57	1.98	6.61	1.29	0.0233	InVivo/SM>InVivo/FM
tr|Q32PD3|Q32PD3_BOVIN	SEC62 protein (Fragment) OS=Bos taurus GN=SEC62 PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.87	90628.06	40294.46	41710.48	14846.61	2.17	12.03	0.43	11.26	0.45	0.0240	
tr|E1BHG5|E1BHG5_BOVIN	Uncharacterized protein OS=Bos taurus PE=4 SV=2	Successfully Matured: In Vivo	2	2	1.98	286626.02	131798.88	122584.67	37566.33	3.00	13.11	0.70	12.09	0.43	0.0243	InVivo/FM>InVitro/FM
sp|Q04173|GDS1_BOVIN	Rap1 GTPase-GDP dissociation stimulator 1 OS=Bos taurus GN=RAP1GDS1 PE=1 SV=1 [sp|Q04173|GDS1_BOVIN,tr|F6RPT3|F6RPT3_BOVIN,tr|Q2KIA8|Q2KIA8_BOVIN]	Successfully Matured: In Vivo	3	3	2.88	12823.72	6779.25	5381.82	5000.93	3.47	10.03	0.57	8.12	1.43	0.0245	
sp|P0C0S4|H2AZ_BOVIN	Histone H2A.Z OS=Bos taurus GN=H2AFZ PE=1 SV=2 [sp|P0C0S4|H2AZ_BOVIN,sp|Q32LA7|H2AV_BOVIN]	Successfully Matured: In Vivo	8	5	7.76	1976335.93	1031316.56	899403.25	464305.68	2.35	15.09	0.51	14.24	0.46	0.0245	
sp|Q0VCR7|C1TM_BOVIN	Monofunctional C1-tetrahydrofolate synthase, mitochondrial OS=Bos taurus GN=MTHFD1L PE=2 SV=2 [sp|Q0VCR7|C1TM_BOVIN,tr|E1B884|E1B884_BOVIN]	Successfully Matured: In Vivo	13	12	12.75	191443.41	104121.20	83349.02	66917.12	2.89	12.76	0.47	11.39	1.00	0.0246	
sp|Q1JPD2|ABHGA_BOVIN	Abhydrolase domain-containing protein 16A OS=Bos taurus GN=ABHD16A PE=2 SV=1 [sp|Q1JPD2|ABHGA_BOVIN,tr|F1MRZ4|F1MRZ4_BOVIN]	Successfully Matured: In Vivo	3	3	2.99	18549.59	10293.81	8278.49	3977.83	2.60	10.41	0.53	9.43	0.59	0.0249	
sp|Q29466-2|VPP1_BOVIN	Isoform 2 of V-type proton ATPase 116 kDa subunit a isoform 1 OS=Bos taurus GN=ATP6V0A1 [sp|Q29466-2|VPP1_BOVIN,sp|Q29466|VPP1_BOVIN,tr|A7Z016|A7Z016_BOVIN,tr|F1MH43|F1MH43_BOVIN,tr|F1MJV0|F1MJV0_BOVIN]	Successfully Matured: In Vivo	4	4	3.98	61283.49	21054.18	27951.27	19281.48	2.15	11.68	0.31	10.78	0.66	0.0250	
sp|Q5E9C2|DAD1_BOVIN	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD1 OS=Bos taurus GN=DAD1 PE=3 SV=3	Successfully Matured: In Vivo	4	4	3.99	400059.75	147373.22	181311.31	104112.82	2.23	13.54	0.34	12.64	0.65	0.0250	InVivo/SM>InVivo/FM
tr|A6QPA4|A6QPA4_BOVIN	COL21A1 protein OS=Bos taurus GN=COL21A1 PE=2 SV=1 [tr|A6QPA4|A6QPA4_BOVIN,tr|E1BGN5|E1BGN5_BOVIN]	Successfully Matured: In Vivo	7	7	6.86	203331.55	88142.52	84340.54	39246.46	3.45	12.77	0.70	11.44	0.83	0.0255	
sp|Q3ZBK2|F213A_BOVIN	Redox-regulatory protein FAM213A OS=Bos taurus GN=FAM213A PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.99	10198.76	7061.45	4342.85	3963.29	3.59	9.71	0.79	8.07	1.08	0.0256	
sp|Q58CP9|STIM1_BOVIN	Stromal interaction molecule 1 OS=Bos taurus GN=STIM1 PE=2 SV=1 [sp|Q58CP9|STIM1_BOVIN,tr|B0JYL7|B0JYL7_BOVIN,tr|G3X721|G3X721_BOVIN]	Successfully Matured: In Vivo	4	4	3.96	34578.23	16320.10	15192.43	6299.58	2.67	11.05	0.48	10.01	0.72	0.0262	InVivo/FM>InVitro/FM
tr|Q8DLU0|Q8DLU0_BOVIN	SLC3A2 protein OS=Bos taurus GN=SLC3A2 PE=2 SV=1 [tr|Q8DLU0|Q8DLU0_BOVIN,tr|Q3T0F0|Q3T0F0_BOVIN,tr|Q5EA54|Q5EA54_BOVIN]	Successfully Matured: In Vivo	4	4	3.93	37535.48	13282.33	15910.10	12307.07	2.94	11.18	0.34	9.62	1.24	0.0266	InVivo/SM>InVivo/FM
sp|P00442|SODC_BOVIN;tr|F1MNQ4|F1MNQ4_BOVIN;tr|Q9TS96|Q9TS96_BOVIN;zz|ZZ_FGCZCont0147|	Superoxide dismutase [Cu-Zn] OS=Bos taurus GN=SOD1 PE=1 SV=2 [sp|P00442|SODC_BOVIN,tr|F1MNQ4|F1MNQ4_BOVIN,tr|Q9TS96|Q9TS96_BOVIN]	Successfully Matured: In Vivo	3	3	2.99	417400.36	198803.76	181740.20	32382.85	2.78	13.48	0.70	12.59	0.24	0.0268	

						Successfully Matured: In Vivo		Successfully Matured: In Vitro			Successfully Matured: In Vivo		Successfully Matured: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|E1BN05|E1BN05_BOVIN	Uncharacterized protein OS=Bos taurus GN=GLDN PE=4 SV=2	Successfully Matured: In Vivo	3	3	2.98	5619.32	6697.64	2295.96	1096.10	8.90	8.64	1.46	3.13	4.32	0.0271	InVivo/SM>InVivo/FM; InVitro/FM>InVivo/FM
tr|Q1JPH2|Q1JPH2_BOVIN;tr|Q5ZCK2|Q5ZCK2_BOVIN	Ras related v-ral simian leukemia viral oncogene homolog A (Fragment) OS=Bos taurus GN=RALA PE=2 SV=1 [tr|Q1JPH2|Q1JPH2_BOVIN,tr|Q3ZCK2|Q3ZCK2_BOVIN]	Successfully Matured: In Vivo	6	2	5.85	78377.10	46286.68	34176.09	25292.60	2.95	11.80	0.66	10.62	0.72	0.0271	
tr|A6QPR1|A6QPR1_BOVIN	PCYOX1 protein OS=Bos taurus GN=PCYOX1 PE=2 SV=1 [tr|A6QPR1|A6QPR1_BOVIN,tr|F1N2K1|F1N2K1_BOVIN]	Successfully Matured: In Vivo	5	5	4.92	26451.39	13060.82	11354.32	8668.61	3.01	10.75	0.60	9.42	0.93	0.0272	
sp|Q8IIG8|RAB18_BOVIN	Ras-related protein Rab-18 OS=Bos taurus GN=RAB18 PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.99	109397.90	43216.35	46320.84	42500.89	3.03	12.23	0.40	10.58	1.32	0.0278	
tr|F1MYW7|F1MYW7_BOVIN	Uncharacterized protein OS=Bos taurus GN=HM13 PE=4 SV=1	Successfully Matured: In Vivo	4	4	3.97	60971.50	25734.11	28373.44	14907.33	2.07	11.65	0.38	10.88	0.52	0.0279	InVivo/SM>InVivo/FM
tr|F1N7F8|F1N7F8_BOVIN	Uncharacterized protein OS=Bos taurus GN=PIGU PE=4 SV=2	Successfully Matured: In Vivo	2	2	1.98	10190.85	6266.93	4193.14	3533.73	4.18	9.69	0.89	7.54	1.56	0.0281	
tr|E1BQ11|E1BQ11_BOVIN	Uncharacterized protein OS=Bos taurus GN=CTNND1 PE=4 SV=2	Successfully Matured: In Vivo	7	7	6.88	126716.58	68596.43	60178.15	16688.84	2.02	12.35	0.46	11.71	0.28	0.0287	
tr|F1MPN0|F1MPN0_BOVIN	Uncharacterized protein OS=Bos taurus GN=FNDC3B PE=4 SV=2	Successfully Matured: In Vivo	9	9	8.94	127459.72	50232.60	55848.41	64884.02	2.59	12.36	0.50	10.94	1.09	0.0296	
sp|P38409|GNA11_BOVIN;tr|E1BA29|E1BA29_BOVIN	Guanine nucleotide-binding protein subunit alpha-11 OS=Bos taurus GN=GNA11 PE=2 SV=2	Successfully Matured: In Vivo	2	2	1.99	59797.54	28101.05	27301.33	23095.43	2.27	11.61	0.46	10.65	0.67	0.0307	
sp|A1A4R1|H2A2C_BOVIN;sp|P0C0S9|H2A1_BOVIN;sp|Q3ZBX9|H2AJ_BOVIN;tr|F2Z4G5|F2Z4G5_BOVIN;tr|F2Z4I6|F2Z4I6_BOVIN;tr|F2Z4J1|F2Z4J1_BOVIN;tr|G8JL00|G8JL00_BOVIN	Histone H2A type 2-C OS=Bos taurus GN=HIST2H2AC PE=2 SV=1 [sp|A1A4R1|H2A2C_BOVIN,sp|P0C0S9|H2A1_BOVIN,sp|Q3ZBX9|H2AJ_BOVIN,tr|F2Z4G5|F2Z4G5_BOVIN,tr|F2Z4I6|F2Z4I6_BOVIN]	Successfully Matured: In Vivo	13	3	12.62	4086200.95	2288291.45	1882751.06	1007213.77	2.28	15.81	0.48	14.97	0.54	0.0308	
tr|A6H7H3|A6H7H3_BOVIN	Estradiol 17-beta-dehydrogenase 12 OS=Bos taurus GN=LOC789567 PE=2 SV=1	Successfully Matured: In Vivo	4	4	3.98	214631.30	125859.04	94278.95	53458.76	2.84	12.84	0.57	11.70	0.79	0.0315	InVivo/SM>InVivo/FM
sp|Q9TU34|ITPR1_BOVIN;sp|Q8WN96|ITPR2_BOVIN;tr|E1BKE7|E1BKE7_BOVIN;tr|F1N137|F1N137_BOVIN	Inositol 1,4,5-trisphosphate receptor type 1 OS=Bos taurus GN=ITPR1 PE=1 SV=1	Successfully Matured: In Vivo	5	1	4.88	2476.00	2140.80	1021.25	435.75	5.25	8.11	1.09	6.61	0.70	0.0318	
sp|Q3SZB4|ACADM_BOVIN	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Bos taurus GN=ACADM PE=2 SV=1	Successfully Matured: In Vivo	20	20	19.77	685760.88	351437.69	316111.40	143276.44	2.23	14.03	0.50	13.25	0.44	0.0320	
sp|A7MB45|ACSS3_BOVIN	Acyl-CoA synthetase short-chain family member 3, mitochondrial OS=Bos taurus GN=ACSS3 PE=2 SV=1 [sp|A7MB45|ACSS3_BOVIN,tr|F1MQV8|F1MQV8_BOVIN]	Successfully Matured: In Vivo	7	7	6.86	160135.30	151834.80	66671.20	33371.94	5.20	12.31	0.95	10.48	1.25	0.0322	
tr|F1MNI5|F1MNI5_BOVIN;sp|O62664|PGH1_BOVIN	Prostaglandin G/H synthase 2 OS=Bos taurus GN=PTGS2 PE=4 SV=1	Successfully Matured: In Vivo	9	9	8.90	101594.70	134070.67	39453.09	807.94	86.03	10.50	2.43	7.62	0.58	0.0324	InVivo/SM>InVivo/FM
tr|F1MWH2|F1MWH2_BOVIN	Adenosylhomocysteinase OS=Bos taurus GN=AHCYL1 PE=1 SV=2	Successfully Matured: In Vivo	6	4	5.84	29162.90	5932.40	13338.80	11516.00	2.01	10.96	0.20	10.04	0.77	0.0330	
tr|Q3T018|Q3T018_BOVIN	Chromosome 14 open reading frame 1 ortholog OS=Bos taurus GN=C10H14ORF1 PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.95	35745.84	20037.30	15272.06	10682.72	3.22	11.06	0.54	9.51	1.24	0.0334	
tr|A6QPX8|A6QPX8_BOVIN	RAB12 protein OS=Bos taurus GN=RAB12 PE=2 SV=1	Successfully Matured: In Vivo	3	1	2.98	8228.78	4693.27	3408.83	1813.70	3.87	9.58	0.56	7.81	1.44	0.0334	
tr|A2VE72|A2VE72_BOVIN	Family with sequence similarity 8, member A1 OS=Bos taurus GN=FAM8A1 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.92	17167.05	8469.90	6422.87	3714.10	8.18	10.36	0.44	6.14	3.66	0.0338	
sp|Q2HJF8|MIRO1_BOVIN	Mitochondrial Rho GTPase 1 OS=Bos taurus GN=RHOT1 PE=2 SV=1 [sp|Q2HJF8|MIRO1_BOVIN,tr|G1K237|G1K237_BOVIN]	Successfully Matured: In Vivo	2	2	1.99	10075.01	7535.78	4360.10	2284.15	3.40	9.71	0.70	8.39	0.92	0.0339	
tr|F1N2P6|F1N2P6_BOVIN	Uncharacterized protein OS=Bos taurus GN=FKBP8 PE=4 SV=1 [tr|F1N2P6|F1N2P6_BOVIN,tr|Q05B91|Q05B91_BOVIN]	Successfully Matured: In Vivo	4	4	3.93	68230.20	26113.85	29497.73	25233.47	2.73	11.77	0.36	10.34	1.20	0.0344	
tr|G5E5T5|G5E5T5_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=1	Successfully Matured: In Vivo	5	4	4.94	26307.10	35453.46	10459.30	1296.05	24.50	9.84	2.07	7.05	1.31	0.0346	
sp|P06623|CN37_BOVIN	2',3'-cyclic-nucleotide 3'-phosphodiesterase OS=Bos taurus GN=CNP PE=2 SV=2	Successfully Matured: In Vivo	12	12	11.76	115440.30	97829.36	46586.89	17060.91	6.10	11.89	1.20	10.28	0.76	0.0354	InVivo/SM>InVivo/FM
sp|P31800|QCR1_BOVIN	Cytochrome b-c1 complex subunit 1, mitochondrial OS=Bos taurus GN=UQCRC1 PE=1 SV=2 [sp|P31800|QCR1_BOVIN,tr|G1K1X0|G1K1X0_BOVIN]	Successfully Matured: In Vivo	19	19	18.56	809082.07	414356.19	376826.28	146762.03	2.14	14.19	0.50	13.47	0.40	0.0356	
tr|Q8E341|Q8E341_BOVIN	Translocase of outer mitochondrial membrane 70 OS=Bos taurus GN=TOMM70A PE=2 SV=1	Successfully Matured: In Vivo	10	9	9.84	152011.71	65628.01	70719.65	42312.63	2.08	12.56	0.40	11.76	0.59	0.0364	
tr|D3K0R6|D3K0R6_BOVIN	Plasma membrane Ca2+-ATPase isoform 4xb (Fragment) OS=Bos taurus GN=PMCA4 PE=2 SV=1	Successfully Matured: In Vivo	11	1	10.83	2029.99	1058.87	905.33	387.82	2.59	8.17	0.63	7.25	0.54	0.0366	
tr|F6QS88|F6QS88_BOVIN	Uncharacterized protein OS=Bos taurus GN=EPHX2 PE=4 SV=1 [tr|F6QS88|F6QS88_BOVIN,tr|Q17QK4|Q17QK4_BOVIN]	Successfully Matured: In Vivo	12	12	11.67	155971.79	82157.15	67998.54	51245.42	2.85	12.52	0.58	11.19	1.05	0.0376	
tr|F1MGK3|F1MGK3_BOVIN	Uncharacterized protein OS=Bos taurus GN=ERLEC1 PE=4 SV=2	Successfully Matured: In Vivo	2	2	1.93	25521.29	10894.04	11730.00	8522.01	2.17	10.77	0.40	9.85	0.74	0.0392	
sp|P46194|CP19A_BOVIN	Aromatase OS=Bos taurus GN=CYP19A1 PE=2 SV=3 [sp|P46194|CP19A_BOVIN,tr|F2Z4F2|F2Z4F2_BOVIN]	Successfully Matured: In Vivo	11	11	10.90	102234.54	108977.21	42148.31	7347.69	6.52	11.69	1.19	10.25	0.54	0.0393	InVivo/SM>InVivo/FM
tr|Q2KJC7|Q2KJC7_BOVIN	Periostin variant 7 OS=Bos taurus GN=POSTN PE=2 SV=1	Successfully Matured: In Vivo	8	1	7.95	4157.02	5520.51	1612.06	40.51	173.31	7.09	4.05	1.84	2.56	0.0397	
sp|A6QPI6|TOM22_BOVIN	Mitochondrial import receptor subunit TOM22 homolog OS=Bos taurus GN=TOMM22 PE=2 SV=1 [sp|A6QPI6|TOM22_BOVIN,tr|E2GEZ1|E2GEZ1_BOVIN]	Successfully Matured: In Vivo	8	8	7.96	167988.91	132038.30	73308.70	37397.35	3.32	12.49	0.76	11.30	0.77	0.0401	
tr|Q2LDY9|Q2LDY9_BOVIN	Prothymosin alpha OS=Bos taurus GN=PTMA PE=4 SV=1	Successfully Matured: In Vivo	9	9	8.87	541367.84	236690.22	228417.65	169254.60	3.17	13.80	0.49	12.02	1.56	0.0407	
tr|F1MV07|F1MV07_BOVIN	Uncharacterized protein OS=Bos taurus GN=SEC24D PE=4 SV=2	Successfully Matured: In Vivo	2	2	1.99	12173.92	5809.27	5518.81	8594.43	2.35	9.98	0.58	8.32	1.41	0.0407	
tr|B0JYK2|B0JYK2_BOVIN	NPC1 protein OS=Bos taurus GN=NPC1 PE=2 SV=1 [tr|B0JYK2|B0JYK2_BOVIN,tr|Q9GLC9|Q9GLC9_BOVIN]	Successfully Matured: In Vivo	7	7	6.92	145362.81	63357.44	66930.18	45354.96	2.16	12.51	0.41	11.61	0.72	0.0413	InVivo/SM>InVivo/FM
sp|Q32LG3|MDHM_BOVIN	Malate dehydrogenase, mitochondrial OS=Bos taurus GN=MDH2 PE=1 SV=1	Successfully Matured: In Vivo	37	36	36.45	13616154.45	9754381.83	6089726.71	1847748.97	2.81	16.91	0.71	16.03	0.40	0.0413	
tr|F1MKG2|F1MKG2_BOVIN	Uncharacterized protein OS=Bos taurus GN=COL6A2 PE=4 SV=2	Successfully Matured: In Vivo	2	2	1.95	7160.43	3672.41	2745.75	1555.04	6.54	9.44	0.60	5.49	3.58	0.0413	InVivo/FM>InVitro/FM
tr|F1MCN8|F1MCN8_BOVIN	Uncharacterized protein OS=Bos taurus GN=AMACR PE=4 SV=2 [tr|F1MCN8|F1MCN8_BOVIN,tr|Q1485P|Q1485P_BOVIN]	Successfully Matured: In Vivo	6	6	5.85	78981.98	55913.64	34453.64	22850.74	3.17	11.80	0.64	10.36	1.16	0.0414	
sp|Q28065|C4BPA_BOVIN	C4b-binding protein alpha chain OS=Bos taurus GN=C4BPA PE=2 SV=1	Successfully Matured: In Vivo	15	10	14.61	182729.93	119276.23	80469.70	98723.67	2.92	12.59	0.79	10.95	1.28	0.0419	InVivo/SM>InVivo/FM
tr|A7E3R8|A7E3R8_BOVIN	Transmembrane protein 109 OS=Bos taurus GN=TMEM109 PE=2 SV=1 [tr|A7E3R8|A7E3R8_BOVIN,tr|Q29RH9|Q29RH9_BOVIN]	Successfully Matured: In Vivo	2	2	1.99	47405.56	22220.76	22016.85	14278.87	2.12	11.38	0.44	10.55	0.64	0.0427	InVivo/SM>InVivo/FM
sp|A6QPN6|GILT_BOVIN	Gamma-interferon-inducible lysosomal thiol reductase OS=Bos taurus GN=IFI30 PE=2 SV=1 [sp|A6QPN6|GILT_BOVIN,tr|F1MAU3|F1MAU3_BOVIN]	Successfully Matured: In Vivo	2	2	1.99	12689.56	20001.45	5174.91	373.79	29.79	8.76	1.98	6.42	0.90	0.0429	
tr|E1BLT3|E1BLT3_BOVIN	Uncharacterized protein OS=Bos taurus GN=SPTBN4 PE=4 SV=1	Successfully Matured: In Vivo	2	1	1.91	14727.29	6682.74	6265.38	2724.62	3.08	10.20	0.47	8.82	1.20	0.0432	
tr|Q148M1|Q148M1_BOVIN	Splicing factor, arginine/serine-rich 11 OS=Bos taurus GN=SFRS11 PE=2 SV=1	Successfully Matured: In Vivo	3	3	2.97	16853.13	7535.60	7619.13	7542.11	2.34	10.33	0.49	9.21	0.93	0.0442	
tr|F1MRZ8|F1MRZ8_BOVIN	Uncharacterized protein OS=Bos taurus GN=PLEK PE=4 SV=2	Successfully Matured: In Vivo	2	2	1.99	4840.49	8962.32	2066.12	334.24	20.13	7.77	1.91	3.49	3.53	0.0442	
tr|A6QQT5|A6QQT5_BOVIN	SSRP1 protein OS=Bos taurus GN=SSRP1 PE=2 SV=1 [tr|A6QQT5|A6QQT5_BOVIN,tr|F6QYV9|F6QYV9_BOVIN]	Successfully Matured: In Vivo	4	4	3.94	36606.12	21593.66	16558.80	14178.33	2.50	11.08	0.54	9.93	0.94	0.0443	
tr|F2Z4I9|F2Z4I9_BOVIN	Magmas-like protein OS=Bos taurus GN=PAM16 PE=4 SV=1	Successfully Matured: In Vivo	2	2	1.89	14277.47	8856.64	6311.72	1247.99	2.82	10.05	0.77	9.20	0.24	0.0444	
sp|Q58DA0|PSMD4_BOVIN	26S proteasome non-ATPase regulatory subunit 4 OS=Bos taurus GN=PSMD4 PE=2 SV=1	Successfully Matured: In Vivo	9	9	8.92	506917.88	277660.99	214061.08	56020.61	3.39	13.60	0.92	12.56	0.36	0.0459	
tr|A1XEE1|A1XEE1_BOVIN	RPLP2 OS=Bos taurus PE=3 SV=1	Successfully Matured: In Vivo	2	1	1.99	344233.33	86759.16	155997.94	130706.63	2.12	13.42	0.23	12.38	0.96	0.0468	
sp|Q0VCP1|KAD4_BOVIN	Adenylate kinase 4, mitochondrial OS=Bos taurus GN=AK4 PE=2 SV=1	Successfully Matured: In Vivo	2	2	1.90	8847.67	5549.36	3911.33	4372.18	2.83	9.61	0.67	7.94	1.45	0.0468	
tr|E1BNI8|E1BNI8_BOVIN	Uncharacterized protein OS=Bos taurus GN=NT5DC2 PE=3 SV=2	Successfully Matured: In Vivo	5	5	4.91	41024.16	19549.48	18581.99	11574.80	2.35	11.21	0.56	10.27	0.70	0.0472	

						Successfully Matured: In Vivo		Successfully Matured: In Vitro			Successfully Matured: In Vivo		Successfully Matured: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
sp|P50291|FST_BOVIN	Follistatin OS=Bos taurus GN=FST PE=2 SV=2	Successfully Matured: In Vivo	9	9	8.84	174681.48	51841.50	78675.07	91042.17	2.21	12.73	0.26	11.30	1.34	0.0473	
sp|Q29RK1|CISY_BOVIN	Citrate synthase, mitochondrial OS=Bos taurus GN=CS PE=1 SV=1	Successfully Matured: In Vivo	9	9	8.75	781916.06	421070.13	365258.64	167388.48	2.14	14.15	0.52	13.41	0.49	0.0475	
sp|F1N4E5|TOP1_BOVIN	Torsin-1A-interacting protein 1 OS=Bos taurus GN=TOR1AIP1 PE=3 SV=2	Successfully Matured: In Vivo	3	3	2.91	48453.44	32494.61	22068.74	21976.25	2.52	11.31	0.65	10.18	0.88	0.0487	
tr|E1B8U3|E1B8U3_BOVIN	Uncharacterized protein OS=Bos taurus GN=GREB1 PE=4 SV=2	Successfully Matured: In Vivo	5	5	4.99	5938.34	3307.72	2558.45	2884.76	3.09	9.16	0.89	7.40	1.44	0.0488	InVivo/FM>InVitro/FM
sp|Q6B411|LYSM_BOVIN	Lysozyme C, milk isozyme OS=Bos taurus PE=2 SV=1	Successfully Matured: In Vivo	2	1	1.90	447.09	616.33	175.09	0.00	Infinity	3.99	3.85	0.00	0.00	0.0493	
sp|Q0V8E7|STRA6_BOVIN	Stimulated by retinoic acid gene 6 protein homolog OS=Bos taurus GN=STRA6 PE=1 SV=1	Successfully Matured: In Vivo	21	21	20.75	4517096.45	1717950.35	2036861.52	1375560.95	2.29	15.93	0.51	14.96	0.78	0.0493	
tr|F1MJQ4|F1MJQ4_BOVIN	Uncharacterized protein OS=Bos taurus GN=P4HA2 PE=4 SV=2	Successfully Matured: In Vivo	18	18	17.94	1078576.88	449570.05	485643.45	245085.93	2.34	14.47	0.60	13.61	0.57	0.0494	
sp|Q2KIS1|RENBP_BOVIN	N-acylglucosamine 2-epimerase OS=Bos taurus GN=RENBP PE=2 SV=2	Successfully Matured: In Vitro	2	2	1.99	6303.73	2088.52	9447.59	3181.97	4.43	9.39	0.36	10.92	0.11	0.0000	InVivo/FM>InVivo/SM
tr|A4FV05|A4FV05_BOVIN	COL18A1 protein OS=Bos taurus GN=COL18A1 PE=2 SV=1	Successfully Matured: In Vitro	8	8	7.75	3926.03	3739.61	19614.82	35211.33	17.59	8.71	0.73	11.74	0.49	0.0001	InVitro/FM>InVivo/FM
tr|F1MBW3|F1MBW3_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=ACSL4 PE=4 SV=1	Successfully Matured: In Vitro	19	17	18.68	104269.33	20037.23	131638.03	118190.80	3.56	12.23	0.18	13.47	0.34	0.0001	InVivo/SM>InVivo/FM; InVitro/FM>InVivo/FM
sp|Q3T0E0|ATOX1_BOVIN	Copper transport protein ATOX1 OS=Bos taurus GN=ATOX1 PE=3 SV=1	Successfully Matured: In Vitro	4	4	3.91	56236.91	11556.08	61630.02	37158.63	2.92	11.61	0.23	12.68	0.25	0.0001	InVivo/FM>InVivo/SM; InVivo/FM>InVitro/FM
tr|A0JBZ9|A0JBZ9_BOVIN	Bucentaur-2 OS=Bos taurus GN=p97bcnt2 PE=2 SV=1	Successfully Matured: In Vitro	7	2	6.82	10723.42	3524.61	13555.30	9263.35	3.54	9.93	0.33	11.21	0.24	0.0001	InVivo/FM>InVivo/SM
tr|F1MU62|F1MU62_BOVIN	Uncharacterized protein OS=Bos taurus GN=CPS17 PE=4 SV=1	Successfully Matured: In Vitro	5	5	4.94	15237.32	2873.25	16135.66	10938.38	2.79	10.31	0.18	11.32	0.29	0.0002	InVivo/FM>InVivo/SM
tr|C3N3M4|C3N3M4_BOVIN;tr|E1B905|E1B905_BOVIN	Uncharacterized protein OS=Bos taurus GN=KCTD12 PE=4 SV=1	Successfully Matured: In Vitro	8	8	7.80	31374.95	10997.43	67186.24	115315.94	6.87	10.99	0.40	12.86	0.52	0.0002	InVivo/SM>InVivo/FM; InVitro/FM>InVivo/FM
sp|Q6H236|PEG3_BOVIN	Paternally-expressed gene 3 protein OS=Bos taurus GN=PEG3 PE=2 SV=1 [sp|Q6H236|PEG3_BOVIN,tr|F1MBU3|F1MBU3_BOVIN]	Successfully Matured: In Vitro	10	10	9.81	2567.32	1686.84	27211.09	96647.93	38.94	8.29	0.86	11.85	0.94	0.0002	InVitro/FM>InVivo/FM
tr|F1MYI2|F1MYI2_BOVIN	Uncharacterized protein OS=Bos taurus GN=HNRNPM PE=4 SV=2	Successfully Matured: In Vitro	50	2	48.34	109211.34	25959.58	114544.79	83509.10	2.74	12.27	0.24	13.27	0.27	0.0003	
tr|A0JNE9|A0JNE9_BOVIN	CTP synthase OS=Bos taurus GN=CTPS PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.94	12648.75	3041.82	13633.04	10403.44	2.85	10.11	0.25	11.15	0.29	0.0003	InVivo/FM>InVivo/SM
sp|O77834|PRDX6_BOVIN	Peroxiredoxin-6 OS=Bos taurus GN=PRDX6 PE=1 SV=3	Successfully Matured: In Vitro	28	27	27.52	900448.72	329306.01	1277927.53	1064469.62	4.08	14.34	0.45	15.79	0.28	0.0003	
sp|Q0P5A1|DCTN3_BOVIN	Dynactin subunit 3 OS=Bos taurus GN=DCTN3 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.98	8091.13	2068.39	9300.02	8680.05	3.12	9.66	0.26	10.78	0.32	0.0003	InVivo/FM>InVivo/SM
sp|Q08E24|SARAF_BOVIN	Store-operated calcium entry-associated regulatory factor OS=Bos taurus GN=TMEM66 PE=2 SV=1	Successfully Matured: In Vitro	6	6	5.69	28804.65	10220.65	35399.74	29375.44	3.40	10.91	0.37	12.15	0.30	0.0004	InVivo/SM>InVivo/FM
sp|O18879|GSTA2_BOVIN	Glutathione S-transferase A2 OS=Bos taurus GN=GSTA2 PE=2 SV=4	Successfully Matured: In Vitro	29	4	28.61	4634.35	2836.54	93404.49	351653.60	75.26	8.92	0.81	12.86	1.35	0.0005	InVitro/FM>InVivo/FM
tr|A6QQX8|A6QQX8_BOVIN	USP39 protein OS=Bos taurus GN=USP39 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.91	8496.72	3932.43	10289.83	5995.95	3.31	9.66	0.47	10.92	0.19	0.0005	InVivo/FM>InVivo/SM
tr|B3VNC2|B3VNC2_BOVIN;tr|A4IFS0|A4IFS0_BOVIN	Artiodactyl-specific sub-telomeric protein OS=Bos taurus GN=LOC508098 PE=2 SV=1	Successfully Matured: In Vitro	8	8	7.90	30776.42	14846.92	106478.65	232392.03	11.85	10.93	0.49	13.28	0.81	0.0006	InVitro/FM>InVivo/FM
tr|G3MYD5|G3MYD5_BOVIN	Uncharacterized protein OS=Bos taurus GN=IVNS1ABP PE=4 SV=1	Successfully Matured: In Vitro	2	2	1.99	932.37	413.69	2807.09	6747.91	10.14	7.45	0.44	9.62	0.78	0.0006	InVivo/FM>InVivo/SM
tr|F1MBN2|F1MBN2_BOVIN	Uncharacterized protein OS=Bos taurus GN=TJP2 PE=4 SV=2	Successfully Matured: In Vitro	3	3	2.93	1555.54	1435.77	8912.93	24593.47	20.38	7.70	0.94	10.76	0.90	0.0008	InVitro/FM>InVivo/FM
sp|Q17R14|MYO1D_BOVIN	Unconventional myosin-Id OS=Bos taurus GN=MYO1D PE=2 SV=1 [sp|Q17R14|MYO1D_BOVIN,tr|F1MJ56|F1MJ56_BOVIN]	Successfully Matured: In Vitro	4	4	3.80	9216.42	2885.06	11570.44	10584.03	3.51	9.77	0.39	11.03	0.37	0.0008	InVivo/FM>InVivo/SM
sp|Q3T0T1|PSB10_BOVIN	Proteasome subunit beta type-10 OS=Bos taurus GN=PSMB10 PE=1 SV=1	Successfully Matured: In Vitro	5	5	4.96	36119.01	12259.66	36588.12	19528.38	2.58	11.14	0.37	12.12	0.22	0.0010	InVivo/FM>InVivo/SM
tr|F1MPJ8|F1MPJ8_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=NPEPL1 PE=4 SV=2 [tr|F1MPJ8|F1MPJ8_BOVIN,tr|Q3SWX3|Q3SWX3_BOVIN]	Successfully Matured: In Vitro	2	2	1.98	1909.19	839.96	2707.41	3514.15	4.10	8.18	0.41	9.57	0.46	0.0010	InVivo/FM>InVivo/SM
sp|Q3SX44|DDAH2_BOVIN	N(G),N(G)-dimethylarginine dimethylaminohydrolase 2 OS=Bos taurus GN=DDAH2 PE=2 SV=1	Successfully Matured: In Vitro	5	4	4.91	32679.06	9843.93	30986.59	14810.34	2.34	11.04	0.34	11.92	0.19	0.0011	InVitro/SM>InVitro/FM
sp|Q2KJ38|F110B_BOVIN	Protein FAM110B OS=Bos taurus GN=FAM110B PE=2 SV=1 [sp|Q2KJ38|F110B_BOVIN,tr|E1BF54|E1BF54_BOVIN]	Successfully Matured: In Vitro	3	3	2.89	646.04	660.39	2862.59	6346.53	15.42	6.78	0.98	9.64	0.92	0.0014	InVitro/FM>InVivo/FM
tr|F1N2X7|F1N2X7_BOVIN;tr|REV_F1N2X7|REV_F1N2X7_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=MLLT4 PE=4 SV=2	Successfully Matured: In Vitro	17	17	16.58	54260.66	23838.44	68594.43	57611.29	3.52	11.49	0.54	12.81	0.31	0.0014	InVivo/FM>InVivo/SM
tr|E1BGF3|E1BGF3_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=HCFC1 PE=4 SV=2	Successfully Matured: In Vitro	7	7	6.97	27741.30	10137.90	24666.33	10848.74	2.11	10.88	0.33	11.65	0.20	0.0019	
tr|Q3T0F9|Q3T0F9_BOVIN	Growth factor receptor-bound protein 2 OS=Bos taurus GN=GRB2 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.97	4293.65	1338.90	4757.80	6147.27	2.95	9.02	0.32	10.06	0.41	0.0021	InVivo/FM>InVivo/SM
sp|Q58DH8|FEN1_BOVIN	Flap endonuclease 1 OS=Bos taurus GN=FEN1 PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.87	10549.70	4605.89	11743.56	6590.35	2.94	9.85	0.55	11.02	0.21	0.0022	InVivo/FM>InVivo/SM
sp|A4FUF0|GLYR1_BOVIN	Putative oxidoreductase GLYR1 OS=Bos taurus GN=GLYR1 PE=2 SV=1 [sp|A4FUF0|GLYR1_BOVIN,tr|M5FK22|M5FK22_BOVIN]	Successfully Matured: In Vitro	2	2	1.99	6029.65	2347.43	5727.53	3185.43	2.33	9.34	0.38	10.22	0.25	0.0025	
tr|Q08ES8|Q08ES8_BOVIN	Tubulin tyrosine ligase-like family, member 12 OS=Bos taurus GN=TTLL12 PE=2 SV=1	Successfully Matured: In Vitro	13	1	12.77	912.37	2038.81	4174.98	15707.84	15.74	2.14	3.96	9.93	0.83	0.0026	InVitro/FM>InVivo/FM
sp|P0C0T1|ITPK1_BOVIN	Inositol-tetrakisphosphate 1-kinase OS=Bos taurus GN=ITPK1 PE=1 SV=1	Successfully Matured: In Vitro	8	8	7.92	40797.35	24997.29	72351.37	76747.36	5.42	11.05	0.94	12.95	0.34	0.0028	InVitro/FM>InVivo/FM
tr|F1MCG5|F1MCG5_BOVIN	Uncharacterized protein OS=Bos taurus GN=TAF2 PE=3 SV=1 [tr|F1MCG5|F1MCG5_BOVIN,tr|F1N2T8|F1N2T8_BOVIN,tr|Q32S33|Q32S33_BOVIN,tr|Q8SQ31|Q8SQ31_BOVIN]	Successfully Matured: In Vitro	2	2	1.91	470.22	520.32	2014.72	7368.88	14.84	6.30	1.27	9.20	0.86	0.0029	InVitro/FM>InVivo/FM
sp|P23196|APEX1_BOVIN	DNA-(apurinic or apyrimidinic site) lyase OS=Bos taurus GN=APEX1 PE=1 SV=2	Successfully Matured: In Vitro	13	13	12.86	462103.68	147945.30	409885.16	126463.38	2.11	13.68	0.40	14.47	0.13	0.0029	
sp|Q5E9D5|DEST_BOVIN	Destrin OS=Bos taurus GN=DSTN PE=2 SV=3	Successfully Matured: In Vitro	5	3	4.78	59854.81	31300.62	77010.22	58482.23	3.58	11.53	0.71	12.94	0.26	0.0031	InVivo/FM>InVivo/SM
tr|E1BA93|E1BA93_BOVIN	Uncharacterized protein OS=Bos taurus GN=SYNPO PE=4 SV=2	Successfully Matured: In Vitro	15	15	14.52	41165.27	15659.46	52735.47	86236.66	3.59	11.26	0.39	12.48	0.53	0.0033	InVivo/FM>InVivo/SM; InVivo/FM>InVitro/FM
tr|Q17QL4|Q17QL4_BOVIN	Melanoma antigen family D, 2 OS=Bos taurus GN=MAGED2 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.90	3319.80	3129.87	6725.27	7027.72	6.31	8.34	1.16	10.59	0.38	0.0034	InVivo/FM>InVivo/SM
tr|A6QLN6|A6QLN6_BOVIN;tr|F1MYM9|F1MYM9_BOVIN	MYH11 protein OS=Bos taurus GN=MYH11 PE=2 SV=1 [tr|A6QLN6|A6QLN6_BOVIN,tr|F1MYM9|F1MYM9_BOVIN]	Successfully Matured: In Vitro	11	1	10.59	85.88	192.04	335.79	1015.38	13.18	1.35	3.02	7.29	1.13	0.0034	InVitro/SM>InVitro/FM
sp|A4IF69|NHLC2_BOVIN	NHL repeat-containing protein 2 OS=Bos taurus GN=NHLRC2 PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.96	2068.07	1215.13	2866.28	4103.74	3.94	8.18	0.62	9.61	0.47	0.0035	InVivo/FM>InVitro/FM
sp|P13696|PEBP1_BOVIN	Phosphatidylethanolamine-binding protein 1 OS=Bos taurus GN=PEBP1 PE=1 SV=2	Successfully Matured: In Vitro	16	16	15.82	1654006.38	545354.71	1461036.94	727429.09	2.09	14.96	0.37	15.73	0.21	0.0035	InVivo/FM>InVivo/SM
tr|F1N556|F1N556_BOVIN	Ubiquitin carboxyl-terminal hydrolase (Fragment) OS=Bos taurus GN=USP7 PE=3 SV=2 [tr|F1N556|F1N556_BOVIN,tr|M5FK76|M5FK76_BOVIN]	Successfully Matured: In Vitro	3	3	2.79	11860.39	4514.04	10646.80	5406.61	2.13	10.02	0.38	10.81	0.21	0.0035	InVivo/FM>InVivo/SM
sp|Q05588|UPAR_BOVIN	Urokinase plasminogen activator surface receptor OS=Bos taurus GN=PLAUR PE=2 SV=1	Successfully Matured: In Vitro	8	8	7.87	14295.85	8009.23	30102.02	65148.60	6.69	10.13	0.56	11.93	0.81	0.0037	InVivo/FM>InVivo/SM
tr|F1MMT2|F1MMT2_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=LAMA2 PE=4 SV=2	Successfully Matured: In Vitro	4	4	3.78	347.34	229.05	1290.23	4646.12	12.76	6.30	0.83	8.67	1.03	0.0041	
sp|Q0III3|DC1I2_BOVIN	Cytoplasmic dynein 1 intermediate chain 2 OS=Bos taurus GN=DYNC1I2 PE=1 SV=1	Successfully Matured: In Vitro	7	7	6.86	123034.34	30315.70	108413.29	72384.00	2.10	12.38	0.29	13.12	0.30	0.0042	
tr|F6QAT4|F6QAT4_BOVIN	Uncharacterized protein OS=Bos taurus GN=REPS1 PE=4 SV=1	Successfully Matured: In Vitro	2	2	1.89	6728.85	2050.41	7753.49	9253.93	3.12	9.46	0.36	10.55	0.50	0.0043	InVivo/FM>InVivo/SM
sp|Q3T046|BDH2_BOVIN	3-hydroxybutyrate dehydrogenase type 2 OS=Bos taurus GN=BDH2 PE=2 SV=1 [sp|Q3T046|BDH2_BOVIN,tr|F1MLA4|F1MLA4_BOVIN]	Successfully Matured: In Vitro	2	2	1.98	2672.84	1427.01	3593.47	4726.77	3.80	8.44	0.64	9.83	0.47	0.0044	InVitro/SM>InVitro/FM
tr|A4FV09|A4FV09_BOVIN	PANK4 protein OS=Bos taurus GN=PANK4 PE=2 SV=1 [tr|A4FV09|A4FV09_BOVIN,tr|F1MLD0|F1MLD0_BOVIN]	Successfully Matured: In Vitro	2	2	1.82	771.53	483.52	1210.44	1906.40	4.63	7.20	0.59	8.72	0.65	0.0046	InVivo/FM>InVivo/SM
sp|Q0VCI1|OSGEP_BOVIN	Probable tRNA N6-adenosine threonylcarbamoyltransferase OS=Bos taurus GN=OSGEP PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.97	13092.81	5374.61	11780.95	4504.37	2.14	10.10	0.44	10.92	0.16	0.0046	InVivo/FM>InVivo/SM

						Successfully Matured: In Vivo		Successfully Matured: In Vitro			Successfully Matured: In Vivo		Successfully Matured: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|E1B7Q0|E1B7Q0_BOVIN	Protein Hook homolog 3 OS=Bos taurus GN=HOOK3 PE=4 SV=1	Successfully Matured: In Vitro	4	4	3.83	6744.82	2788.76	7745.88	8398.10	3.08	9.43	0.47	10.56	0.46	0.0048	InVivo/FM>InVivo/SM
sp|Q5E9G0|GSTA4_BOVIN	Glutathione S-transferase A4 OS=Bos taurus GN=GSTA4 PE=2 SV=1 [sp|Q5E9G0|GSTA4_BOVIN,tr|Q321L24|Q321L24_BOVIN]	Successfully Matured: In Vitro	2	2	1.85	33673.73	8760.92	33077.55	28471.27	2.48	11.08	0.30	11.97	0.41	0.0049	
tr|F1MBF0|F1MBF0_BOVIN	Eukaryotic translation initiation factor 2 subunit 1 OS=Bos taurus GN=EIF2A PE=4 SV=1	Successfully Matured: In Vitro	10	10	9.76	49582.85	19657.05	47291.69	24319.22	2.34	11.43	0.47	12.34	0.24	0.0050	InVivo/FM>InVivo/SM
sp|A4FUE7|ZC21A_BOVIN	Zinc finger C2HC domain-containing protein 1A OS=Bos taurus GN=ZC2HC1A PE=2 SV=1 [sp|A4FUE7|ZC21A_BOVIN,tr|G1K1W5|G1K1W5_BOVIN]	Successfully Matured: In Vitro	2	2	1.93	1484.12	413.18	2656.11	5434.66	5.54	7.96	0.30	9.47	0.83	0.0051	InVivo/FM>InVivo/SM
sp|P61955|SUMO2_BOVIN	Small ubiquitin-related modifier 2 OS=Bos taurus GN=SUMO2 PE=3 SV=1 [sp|P61955|SUMO2_BOVIN,tr|G3N303|G3N303_BOVIN,tr|G5E5Y5|G5E5Y5_BOVIN]	Successfully Matured: In Vitro	2	2	1.96	335173.83	149784.22	357201.99	364628.96	2.76	13.34	0.45	14.37	0.41	0.0051	
tr|F1MSQ6|F1MSQ6_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=NEFH PE=3 SV=2	Successfully Matured: In Vitro	5	1	4.90	30544.16	11159.45	32252.88	32470.23	2.74	10.95	0.45	11.97	0.40	0.0053	InVivo/FM>InVivo/SM
sp|Q8HXY9|CFDP1_BOVIN	Craniofacial development protein 1 OS=Bos taurus GN=CFDP1 PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.968	24560.33555	5007.058816	21413.07565	16954.69452	2.071750573	10.78450136	0.212063868	11.4823495	0.353341703	0.0053	InVivo/FM>InVivo/SM
sp|A6QP17|ERAP2_BOVIN	Endoplasmic reticulum aminopeptidase 2 OS=Bos taurus GN=ERAP2 PE=2 SV=1	Successfully Matured: In Vitro	4	4	3.979	2737.824677	1706.749089	3333.724717	2210.423201	3.29866813	8.405162094	0.766464744	9.772657824	0.28208094	0.0057	InVivo/FM>InVivo/SM
sp|A2VE99|SEPT1_BOVIN;sp|Q5RZN0|SEPT6_BOVIN	Septin-11 OS=Bos taurus GN=SEPT11 PE=2 SV=1	Successfully Matured: In Vitro	9	8	8.825	194861.2915	81385.79032	190062.2152	103594.1177	2.42280172	12.77370364	0.54632785	13.73840969	0.223194103	0.0064	
sp|O02751|CFDP2_BOVIN	Craniofacial development protein 2 OS=Bos taurus GN=CFDP2 PE=1 SV=2	Successfully Matured: In Vitro	21	16	20.398	354299.9657	131390.3243	308764.6069	119105.9973	2.037401586	13.40053774	0.448956926	14.17198178	0.163332609	0.0069	
tr|G3N0W8|G3N0W8_BOVIN	Uncharacterized protein OS=Bos taurus GN=PURB PE=4 SV=1	Successfully Matured: In Vitro	4	4	3.891	61791.92688	30536.1222	61427.76561	47586.9034	2.478542594	11.62115721	0.52235581	12.59433122	0.306937947	0.0071	
sp|Q24K16|ZADH2_BOVIN	Zinc-binding alcohol dehydrogenase domain-containing protein 2 OS=Bos taurus GN=ZADH2 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.8	2636.571644	806.8165825	3195.296659	6674.69853	3.340162337	8.5316596	0.313607529	9.607432213	0.597125534	0.0073	InVivo/FM>InVivo/SM
tr|A7E3V0|A7E3V0_BOVIN	Sequestosome 1 OS=Bos taurus GN=SQSTM1 PE=2 SV=1 [tr|A7E3V0|A7E3V0_BOVIN,tr|F1MIE2|F1MIE2_BOVIN,tr|Q32PJ9|Q32PJ9_BOVIN]	Successfully Matured: In Vitro	2	2	1.994	11645.55508	8621.681762	14620.67763	17865.35211	3.421700071	9.871371968	0.651220709	11.19087032	0.51278408	0.0074	
sp|Q0P518|GET4_BOVIN	Golgi to ER traffic protein 4 homolog OS=Bos taurus GN=GET4 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.983	645.1130813	370.6485555	711.1219639	618.8814812	2.860655693	7.032605551	0.562593409	8.151837916	0.425859291	0.0075	
sp|Q5E9A6|VPS25_BOVIN	Vacuolar protein-sorting-associated protein 25 OS=Bos taurus GN=VPS25 PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.99	5899.276794	2802.144338	5574.666575	3536.179068	2.293999175	9.278900459	0.505943663	10.1793436	0.257740763	0.0076	InVivo/FM>InVivo/SM
tr|Q1RMT6|Q1RMT6_BOVIN	Drebrin 1 OS=Bos taurus GN=DBN1 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.904	6177.198201	3853.53845	9485.073185	16897.68559	4.508307359	9.232625081	0.726566914	10.76262025	0.659926238	0.0083	
tr|Q0P592|Q0P592_BOVIN	Fructosamine 3 kinase related protein OS=Bos taurus GN=FN3KRP PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.884	14838.99459	7625.754228	17395.10438	23141.80622	3.15096302	10.18281688	0.548616749	11.34341402	0.509621145	0.0085	InVivo/FM>InVivo/SM
sp|P10881|LA_BOVIN	Lupus La protein homolog OS=Bos taurus GN=SSB PE=2 SV=2	Successfully Matured: In Vitro	9	9	8.873	180043.0152	64873.3216	158340.341	78633.72596	2.069792344	12.72341316	0.454288206	13.50213785	0.225182401	0.0089	
sp|Q8MJJ7|DCPS_BOVIN	m7GpppX diphosphatase OS=Bos taurus GN=DCPS PE=2 SV=1 [sp|Q8MJJ7|DCPS_BOVIN,tr|A5D7U9|A5D7U9_BOVIN]	Successfully Matured: In Vitro	4	4	3.891	33244.15285	10941.21154	28923.67897	16826.74159	2.039920088	11.05249459	0.380727279	11.78732418	0.290290045	0.0089	
tr|Q17QL8|Q17QL8_BOVIN	Lysophospholipase II OS=Bos taurus GN=LYPLA2 PE=2 SV=1	Successfully Matured: In Vitro	4	4	3.904	57940.44423	24892.09632	53617.0448	33306.60456	2.230287231	11.57133049	0.496881518	12.43422064	0.269930431	0.0092	InVivo/FM>InVivo/SM
tr|E1BME6|E1BME6_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=HK2 PE=3 SV=2	Successfully Matured: In Vitro	33	27	32.45	580112.5143	165469.7292	560093.9663	575814.979	2.411793055	13.92262897	0.342451287	14.77092063	0.438042965	0.0092	InVitro/FM>InVivo/FM
tr|Q0VC96|Q0VC96_BOVIN	Thymocyte nuclear protein 1 OS=Bos taurus GN=THYN1 PE=2 SV=1	Successfully Matured: In Vitro	5	5	4.879	50856.76367	16031.44235	46859.28123	40269.35298	2.237991677	11.47253991	0.41454448	12.28814284	0.339785205	0.0093	
sp|Q58DC0|CPPED_BOVIN	Calcineurin-like phosphoesterase domain-containing protein 1 OS=Bos taurus GN=CPPED1 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.987	15675.62407	5843.653233	16288.11859	16939.21652	2.673128009	10.26017877	0.545681115	11.27366742	0.39213854	0.0097	InVivo/FM>InVivo/SM
sp|Q3SZJ9|PMM2_BOVIN	Phosphomannomutase 2 OS=Bos taurus GN=PMM2 PE=2 SV=1 [sp|Q3SZJ9|PMM2_BOVIN,tr|M5FJZ1|M5FJZ1_BOVIN]	Successfully Matured: In Vitro	7	7	6.861	35677.7631	19390.59263	34648.61428	17098.17491	2.38131327	11.03654195	0.62737055	12.02729722	0.197484637	0.0098	
tr|F1MUM9|F1MUM9_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=ADAR PE=4 SV=2	Successfully Matured: In Vitro	2	2	1.964	661.2591017	552.1522892	860.5739458	1213.061356	3.565598551	6.909017838	0.821489933	8.353717388	0.511650464	0.0103	
sp|Q3MHZ8|LEG9_BOVIN	Galectin-9 OS=Bos taurus GN=LGALS9 PE=2 SV=1 [sp|Q3MHZ8|LEG9_BOVIN,tr|F1MZ12|F1MZ12_BOVIN,tr|Q5E9E0|Q5E9E0_BOVIN]	Successfully Matured: In Vitro	3	3	2.981	2838.367862	2500.096181	5786.00179	11511.20275	6.366551447	8.274228365	1.01016972	10.24765952	0.869123674	0.0107	InVivo/FM>InVivo/SM
tr|A5D7T2|A5D7T2_BOVIN	MGC148871 protein OS=Bos taurus GN=MGC148871 PE=2 SV=1	Successfully Matured: In Vitro	6	6	5.95	12312.86196	5900.712247	15067.00118	21201.16135	3.353155655	9.945860694	0.749677253	11.22874668	0.46657858	0.0117	InVivo/FM>InVivo/SM
sp|Q9TRY0|FKBP4_BOVIN	Peptidyl-prolyl cis-trans isomerase FKBP4 OS=Bos taurus GN=FKBP4 PE=1 SV=4	Successfully Matured: In Vitro	28	2	27.353	26617.05507	9169.943203	24833.63017	24819.43516	2.275994488	10.82233009	0.412561895	11.64223923	0.390117241	0.0121	InVivo/FM>InVivo/SM
sp|Q17Q17|FA49A_BOVIN;sp|Q2KJD3|FA49B_BOVIN	Protein FAM49A OS=Bos taurus GN=FAM49A PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.989	11560.85808	8114.443088	17715.65846	49846.11748	4.481641927	9.847373359	0.703639602	11.2929634	0.714389375	0.0122	InVivo/FM>InVivo/SM
tr|E1BHU1|E1BHU1_BOVIN;tr|A6QP77|A6QP77_BOVIN	Uncharacterized protein OS=Bos taurus GN=TCOF1 PE=4 SV=1	Successfully Matured: In Vitro	12	12	11.678	32927.28938	19178.18909	31343.40635	22381.67838	2.300319724	10.95735726	0.585185901	11.89408036	0.290353265	0.0125	
sp|Q5EAB2|CDK9_BOVIN	Cyclin-dependent kinase 9 OS=Bos taurus GN=CDK9 PE=2 SV=1	Successfully Matured: In Vitro	3	1	2.928	2690.968198	1118.498891	2670.102366	2880.420956	2.483573393	8.525393031	0.399423225	9.4153255	0.47568572	0.0125	
tr|A4FV23|A4FV23_BOVIN	DDX23 protein OS=Bos taurus GN=DDX23 PE=2 SV=1	Successfully Matured: In Vitro	8	6	7.929	15132.18235	6871.103719	16393.09804	21095.52974	2.825009641	10.23133852	0.4717011	11.24907552	0.53779928	0.0130	
sp|Q3ZCK3|BPNT1_BOVIN	3'(2'),5'-bisphosphate nucleotidase 1 OS=Bos taurus GN=BPNT1 PE=2 SV=1	Successfully Matured: In Vitro	5	5	4.868	19187.02845	6323.232093	17059.11701	14491.17033	2.111993748	10.50996926	0.339875906	11.24543133	0.390376711	0.0131	
sp|Q2HJ74|GATM_BOVIN	Glycine amidinotransferase, mitochondrial OS=Bos taurus GN=GATM PE=2 SV=1	Successfully Matured: In Vitro	7	7	6.723	13149.14516	7493.366075	21571.69569	54121.71586	4.919152126	10.01268209	0.678214267	11.50792055	0.808076388	0.0132	
sp|Q0IIM3|HS105_BOVIN	Heat shock protein 105 kDa OS=Bos taurus GN=HSPH1 PE=2 SV=1	Successfully Matured: In Vitro	13	11	12.873	88560.77467	44433.37659	94137.51086	62063.2627	2.738780151	11.90863738	0.770784177	13.0639662	0.27089639	0.0134	
tr|A5D7A2|A5D7A2_BOVIN	GARS protein OS=Bos taurus GN=GARS PE=2 SV=1	Successfully Matured: In Vitro	9	9	8.882	73881.72627	32796.64012	65671.4061	26969.17127	2.088739442	11.79648665	0.560833197	12.62706582	0.181885532	0.0136	
tr|A1A4I8|A1A4I8_BOVIN	Uncharacterized protein OS=Bos taurus GN=XPO7 PE=2 SV=1	Successfully Matured: In Vitro	4	4	3.875	6839.234571	3255.491085	6879.00761	6745.8067	2.525231021	9.404138452	0.584749702	10.38850534	0.391485715	0.0141	
sp|A1L5A6|ADRM1_BOVIN	Proteasomal ubiquitin receptor ADRM1 OS=Bos taurus GN=ADRM1 PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.991	39426.21429	13299.25147	35265.27946	31647.89304	2.131150905	11.21885305	0.398153756	11.97335562	0.385888958	0.0160	
sp|Q8DY9|CASP3_BOVIN;sp|Q3T0P5|CASP6_BOVIN;tr|F1MB04|F1MB04_BOVIN;tr|G3X702|G3X702_BOVIN	Caspase-3 OS=Bos taurus GN=CASP3 PE=2 SV=1 [sp|Q8DY9|CASP3_BOVIN,tr|F1MB04|F1MB04_BOVIN]	Successfully Matured: In Vitro	2	2	1.982	10766.43726	6265.460601	12351.559	5772.106735	3.041977438	9.708071142	0.992376343	11.07708937	0.179751722	0.0162	InVivo/FM>InVivo/SM
sp|A6QL10|EPDR1_BOVIN	Mammalian ependymin-related protein 1 OS=Bos taurus GN=EPDR1 PE=2 SV=1	Successfully Matured: In Vitro	4	4	3.888	162091.0604	71632.56642	151793.9133	126963.7356	2.270118254	12.57819609	0.57888811	13.46754216	0.310553963	0.0164	
tr|Q2KII9|Q2KII9_BOVIN	Dynein, cytoplasmic 1, light intermediate chain 1 OS=Bos taurus GN=DYNC1LI1 PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.978	20713.64286	9028.388107	23967.69083	43331.75595	3.109179892	10.50521128	0.639864106	11.63195859	0.533145316	0.0164	
tr|F1N533|F1N533_BOVIN	Uncharacterized protein OS=Bos taurus GN=DDX18 PE=3 SV=2	Successfully Matured: In Vitro	2	2	1.994	6725.854686	3617.966899	7782.624587	9380.948837	3.087786665	9.337170693	0.727068638	10.53902134	0.513911944	0.0166	
tr|Q0VCH9|Q0VCH9_BOVIN	GLI pathogenesis-related 2 OS=Bos taurus GN=GLIPR2 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.994	12655.21141	6968.407824	12865.67214	17007.73834	2.552108967	10.03509785	0.491150029	10.9736346	0.493822764	0.0167	
sp|Q2TSV8|DTD1_BOVIN	D-tyrosyl-tRNA(Tyr) deacylase 1 OS=Bos taurus GN=DTD1 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.994	5657.647732	1811.863918	5174.221517	5329.567276	2.209270803	9.293646578	0.316885399	10.04719443	0.460846203	0.0167	InVivo/FM>InVivo/SM
tr|F1N4K1|F1N4K1_BOVIN	Uncharacterized protein OS=Bos taurus GN=PEAS PE=4 SV=2	Successfully Matured: In Vitro	4	4	3.837	7420.421931	3814.615349	8447.75096	8494.633051	3.02173706	9.416573057	0.802104029	10.64152499	0.439537264	0.0172	
sp|O46414|FRIH_BOVIN	Ferritin heavy chain OS=Bos taurus GN=FTH1 PE=2 SV=3 [sp|O46414|FRIH_BOVIN,tr|G1K1K0|G1K1K0_BOVIN]	Successfully Matured: In Vitro	4	4	3.89	152164.0059	45523.57358	136787.2906	132288.4276	2.156061891	12.58944847	0.302968804	13.31902631	0.453222956	0.0173	InVitro/FM>InVivo/FM
sp|Q0VBZ9|MRP_BOVIN;tr|G3MY11|G3MY11_BOVIN	MARCKS-related protein OS=Bos taurus GN=MARCKSL1 PE=2 SV=1	Successfully Matured: In Vitro	6	5	5.878	163979.7401	70985.78313	152823.2444	109642.679	2.254792333	12.58708844	0.594522642	13.47786936	0.300831473	0.0173	InVitro/SM>InVitro/FM
tr|F1N2W0|F1N2W0_BOVIN	Prostaglandin reductase 1 OS=Bos taurus GN=PTGR1 PE=4 SV=1	Successfully Matured: In Vitro	19	18	18.684	508658.4377	215377.5751	464953.3684	436024.6746	2.188778321	13.7593425	0.435660827	14.55231275	0.402683791	0.0174	
sp|P48734|CDK1_BOVIN	Cyclin-dependent kinase 1 OS=Bos taurus GN=CDK1 PE=2 SV=2	Successfully Matured: In Vitro	4	2	3.829	3925.068019	2818.775018	4652.803197	8484.654177	3.158199916	8.773907071	0.686313666	9.969747332	0.574277584	0.0174	InVivo/FM>InVivo/SM
tr|A6QR08|A6QR08_BOVIN	GPS1 protein OS=Bos taurus GN=GPS1 PE=2 SV=1 [tr|A6QR08|A6QR08_BOVIN,tr|F1MP10|F1MP10_BOVIN]	Successfully Matured: In Vitro	4	4	3.855	22010.21446	8282.897452	19244.38305	15805.53165	2.046639875	10.62738021	0.416602802	11.3582495	0.358374384	0.0178	InVivo/FM>InVivo/SM
tr|A5PJR3|A5PJR3_BOVIN	Dicarbonyl/L-xylulose reductase OS=Bos taurus GN=DCXR PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.917	12663.23829	8130.881904	14100.08	10357.72906	2.901801462	9.876702026	0.931258102	11.1724473	0.286264085	0.0178	InVivo/FM>InVivo/SM
sp|Q29RL9|TCEA1_BOVIN	Transcription elongation factor A protein 1 OS=Bos taurus GN=TCEA1 PE=2 SV=1 [sp|Q29RL9|TCEA1_BOVIN,tr|F1MIT2|F1MIT2_BOVIN]	Successfully Matured: In Vitro	5	5	4.979	22617.37305	9499.745947	22928.85113	24260.85196	2.567760303	10.59973786	0.625350988	11.59426988	0.410978795	0.0178	InVivo/FM>InVivo/SM

						Successfully Matured: In Vivo		Successfully Matured: In Vitro			Successfully Matured: In Vivo		Successfully Matured: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
sp | Q05443 | LUM_BOVIN	Lumican OS=Bos taurus GN=LUM PE=1 SV=1	Successfully Matured: In Vitro	3	3	2.881	8941.951709	6042.275696	10960.9889	11590.39873	3.321575518	9.499966428	0.963842407	10.91647916	0.459774918	0.0180	InVitro/FM>InVivo/FM
sp | Q3SZ73 | ABHDB_BOVIN	Alpha/beta hydrolase domain-containing protein 11 OS=Bos taurus GN=ABHD11 PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.881	15699.68362	7925.852997	14645.44884	14155.4445	2.24307151	10.24779604	0.521313019	11.10102685	0.389683227	0.0190	
tr | E1BM48 | E1BM48_BOVIN	Uncharacterized protein OS=Bos taurus GN=PRPF31 PE=4 SV=1	Successfully Matured: In Vitro	5	5	4.96	13139.80407	8445.55918	12556.93894	9660.580466	2.301963584	10.00406728	0.679441604	10.97334043	0.297148276	0.0192	
sp | Q3ZLM2 | SGTA_BOVIN	Small glutamine-rich tetratricopeptide repeat-containing protein alpha OS=Bos taurus GN=SGTA PE=2 SV=1	Successfully Matured: In Vitro	7	7	6.849	82358.92385	30479.76056	75723.94975	58383.90848	2.21967368	11.92002313	0.543510052	12.76334938	0.349038821	0.0193	
tr | A4IFH7 | A4IFH7_BOVIN	MAP2K3 protein OS=Bos taurus GN=MAP2K3 PE=2 SV=1	Successfully Matured: In Vitro	4	4	3.828	3174.908299	1550.96981	3278.265841	1506.837163	2.622719547	8.546740904	0.880639397	9.707822453	0.175532848	0.0202	
tr | E1BJX6 | E1BJX6_BOVIN	Uncharacterized protein OS=Bos taurus GN=SCUBE3 PE=4 SV=1	Successfully Matured: In Vitro	2	2	1.994	136.3032668	125.3820442	2706.201013	17305.05734	74.01837098	4.549059562	2.594417931	8.651522992	1.834710803	0.0203	InVitro/FM>InVivo/FM
tr | Q3MHK9 | Q3MHK9_BOVIN	Fascin OS=Bos taurus GN=FSCN1 PE=2 SV=1	Successfully Matured: In Vitro	6	6	5.769	15907.36306	15829.76762	28403.40124	48733.95572	5.385308773	9.865483731	1.175485773	11.79551617	0.957819922	0.0216	InVitro/SM>InVitro/FM
sp | Q3SZD7 | CBR1_BOVIN	Carbonyl reductase [NADPH] 1 OS=Bos taurus GN=CBR1 PE=2 SV=1 [sp | Q3SZD7 | CBR1_BOVIN,tr | G1K231 | G1K231_BOVIN]	Successfully Matured: In Vitro	5	5	4.759	4643.795344	4796.997164	6543.53855	13114.69877	3.945643824	8.795167387	0.880137974	10.28278101	0.77356923	0.0219	
sp | Q2NKS3 | PSMG3_BOVIN	Proteasome assembly chaperone 3 OS=Bos taurus GN=PSMG3 PE=2 SV=1	Successfully Matured: In Vitro	4	4	3.956	15371.62329	10611.11521	15859.13026	16784.35264	2.581655547	10.1074277	0.792782839	11.22006386	0.37861761	0.0221	InVivo/FM>InVivo/SM
tr | Q0VCU3 | Q0VCU3_BOVIN	Cathepsin F OS=Bos taurus GN=CTSF PE=2 SV=1	Successfully Matured: In Vitro	4	4	3.962	676.4695659	652.0815441	2008.336208	10305.86707	9.876333913	6.910215608	0.831143156	8.76639835	1.215918775	0.0226	
tr | G5E6Q2 | G5E6Q2_BOVIN	Uncharacterized protein OS=Bos taurus GN=ZNF358 PE=4 SV=1	Successfully Matured: In Vitro	4	4	3.949	4652.450648	2605.000204	9439.524906	25182.3686	6.395630556	8.956494332	0.752526756	10.607329	1.073401128	0.0226	InVivo/FM>InVivo/SM
tr | E1BJQ1 | E1BJQ1_BOVIN	Uncharacterized protein OS=Bos taurus GN=GLDC PE=3 SV=2	Successfully Matured: In Vitro	3	3	2.929	5890.205922	3105.511493	7217.524902	15267.02665	3.348951589	9.283433801	0.453120598	10.36190287	0.727393526	0.0227	
sp | Q3SYX0 | NDRG1_BOVIN	Protein NDRG1 OS=Bos taurus GN=NDRG1 PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.991	54682.42282	19512.24629	49710.01867	49324.42684	2.182836371	11.54932469	0.368660251	12.30075173	0.473670187	0.0232	InVitro/FM>InVivo/FM
zz | ZZ_FGCZCont0014 | zz | ZZ_FGCZCont0168 |	gi | 346581 | pir | S29094 keratin, type II, component 5, cytoskeletal - sh [zz | ZZ_FGCZCont0014 | zz | ZZ_FGCZCont0168 |]	Successfully Matured: In Vitro	18	1	17.483	41.31646422	60.24528485	80.75280875	213.5672185	5.859949458	2.115533209	2.90413603	5.896922499	0.843932854	0.0233	
sp | Q5EAD2 | SERA_BOVIN	D-3-phosphoglycerate dehydrogenase OS=Bos taurus GN=PHGDH PE=2 SV=3	Successfully Matured: In Vitro	19	19	18.768	591505.9733	326409.0482	590725.0399	547596.7033	2.484589385	13.80075218	0.752683207	14.84023192	0.362079771	0.0238	InVivo/FM>InVivo/SM
sp | Q3SZM1 | MK67I_BOVIN	MKI67 FHA domain-interacting nucleolar phosphoprotein OS=Bos taurus GN=NIFK PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.967	3787.289971	1918.584014	5222.219766	13755.35745	3.935606079	8.813182361	0.565463019	10.02086104	0.799925031	0.0248	
sp | Q2KJA1 | SH3G1_BOVIN;tr | Q08DX1 | Q08DX1_BOVIN	Endophilin-A2 OS=Bos taurus GN=SH3GL1 PE=2 SV=1	Successfully Matured: In Vitro	6	6	5.827	17856.26046	7511.769953	16091.9806	16044.93007	2.13970237	10.40201055	0.472595171	11.17496329	0.413237791	0.0249	InVivo/FM>InVivo/SM
tr | E1BFD5 | E1BFD5_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=CUL4B PE=3 SV=2	Successfully Matured: In Vitro	4	2	3.96	11954.59633	4020.630046	11207.52114	14906.91583	2.294249568	10.03653169	0.337246827	10.80095326	0.52209014	0.0251	
sp | Q2NL31 | MTNA_BOVIN	Methylthioribose-1-phosphate isomerase OS=Bos taurus GN=MRI1 PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.982	3023.772704	2194.741081	4743.202307	13028.87609	4.612265509	8.360787918	1.089044452	9.977402668	0.738029328	0.0251	InVivo/FM>InVivo/SM
tr | Q0V888 | Q0V888_BOVIN	Coatomer protein complex, subunit gamma 1 OS=Bos taurus GN=COPG1 PE=2 SV=1	Successfully Matured: In Vitro	12	1	11.699	278.4017963	168.4781959	940.4220958	2748.694253	11.48814947	6.128392438	0.753253765	8.188912454	1.498886594	0.0252	
tr | G3N266 | G3N266_BOVIN	Uncharacterized protein OS=Bos taurus GN=GFSM1 PE=4 SV=1	Successfully Matured: In Vitro	2	2	1.976	2288.639306	1390.243699	2569.175915	1685.441169	2.939951576	8.122091309	1.086624802	9.482383437	0.248523132	0.0259	
sp | Q29465 | SYYC_BOVIN	Tyrosine--tRNA ligase, cytoplasmic OS=Bos taurus GN=YARS PE=2 SV=4 [sp | Q29465 | SYYC_BOVIN,tr | F1M HM5 | F1MHM5_BOVIN,tr | Q0III5 | Q0III5_BOVIN]	Successfully Matured: In Vitro	4	4	3.923	25165.99282	17498.98491	26302.74217	27679.16464	2.632066832	10.56740462	0.879968133	11.73237979	0.380694662	0.0264	
tr | E1BP50 | E1BP50_BOVIN	Uncharacterized protein OS=Bos taurus GN=TNKS1BP1 PE=4 SV=2	Successfully Matured: In Vitro	4	4	3.972	2870.055371	1599.826388	3150.457401	3925.239104	2.856182913	8.449845296	0.817384335	9.607643717	0.497895071	0.0269	InVivo/FM>InVivo/SM
tr | E1BF55 | E1BF55_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=MAP7D2 PE=4 SV=2	Successfully Matured: In Vitro	7	6	6.771	50323.67385	24854.49469	48730.00301	32467.83142	2.380608023	11.33459672	0.803192275	12.3554218	0.285485219	0.0280	
sp | Q3MHE4 | MSH2_BOVIN	DNA mismatch repair protein Msh2 OS=Bos taurus GN=MSH2 PE=2 SV=1	Successfully Matured: In Vitro	5	5	4.876	13252.29116	5344.138521	12128.41553	10935.42969	2.196163035	10.07587592	0.594413742	10.91533492	0.37499597	0.0283	InVivo/FM>InVivo/SM
tr | E1BAZ4 | E1BAZ4_BOVIN	Hydroxypyruvate isomerase OS=Bos taurus GN=HYI PE=3 SV=2	Successfully Matured: In Vitro	4	4	3.899	3813.920719	2399.856967	4564.413154	5728.356967	3.219134963	8.631143236	1.054119609	10.01772691	0.486953515	0.0284	InVivo/FM>InVivo/SM
tr | Q2HJI9 | Q2HJI9_BOVIN	Ring-box 1 OS=Bos taurus GN=RBX1 PE=4 SV=1	Successfully Matured: In Vitro	4	4	3.988	21444.04882	15057.87908	21904.98874	23158.24077	2.540649498	10.48091569	0.669284575	11.5010382	0.533342503	0.0286	
sp | P79342 | S10AD_BOVIN	Protein S100-A13 OS=Bos taurus GN=S100A13 PE=3 SV=2	Successfully Matured: In Vitro	2	2	1.994	35193.86085	18106.4758	31412.38445	21994.90996	2.088426002	11.02859265	0.621517126	11.85948489	0.318673037	0.0288	
tr | F1MWG7 | F1MWG7_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=TP53BP2 PE=4 SV=2	Successfully Matured: In Vitro	3	3	2.847	6338.444728	5730.80605	6326.202996	5079.031713	2.410082194	9.097170807	0.935597847	10.27956411	0.350996033	0.0294	
tr | A4FUG0 | A4FUG0_BOVIN	SNAPIN protein OS=Bos taurus GN=SNAPIN PE=2 SV=1	Successfully Matured: In Vitro	4	4	3.988	268.8218151	460.2508397	516.1591719	1648.05052	5.738513397	2.798892638	3.861242699	7.552628647	1.124020223	0.0296	
sp | Q2HJ47 | DENR_BOVIN	Density-regulated protein OS=Bos taurus GN=DENR PE=2 SV=1 [sp | Q2HJ47 | DENR_BOVIN,tr | E1BIK7 | E1BIK7_BOVIN]	Successfully Matured: In Vitro	2	2	1.98	15851.26697	8034.707614	16301.70859	15638.73992	2.605827591	10.15948906	0.852825721	11.26398763	0.383525615	0.0297	
sp | Q1LZA3 | ASNS_BOVIN	Asparagine synthetase [glutamine-hydrolyzing] OS=Bos taurus GN=ASNS PE=2 SV=3 [sp | Q1LZA3 | ASNS_BOVIN,tr | A1L588 | A1L588_BOVIN]	Successfully Matured: In Vitro	2	2	1.895	2919.616002	1900.441364	2848.920886	1531.964225	2.374279282	8.423000972	0.90253995	9.516983658	0.225665176	0.0302	
sp | Q865V6 | CAPG_BOVIN	Macrophage-capping protein OS=Bos taurus GN=CAPG PE=2 SV=1	Successfully Matured: In Vitro	5	5	4.985	3887.833229	1704.792082	59359.97026	200606.7333	56.72845755	8.882359464	0.437877505	11.74396978	2.396927863	0.0304	InVitro/FM>InVivo/FM
tr | C9J3R9 | C9J3R9_BOVIN;tr | C9W0W3 | C9W0W3_BOVIN;tr | C9W0Q1 | C9W0Q1_BOVIN;tr | K7QE84 | K7QE84_BOVIN;tr | O46711 | O46711_BOVIN;tr | Q3YJI6 | Q3YJI6_BOVIN;tr | W5XHU3 | W5XHU3_BOVIN	MHC class I antigen (Fragment) OS=Bos taurus GN=BoLA PE=2 SV=1	Successfully Matured: In Vitro	11	4	10.895	8967.101892	2908.956767	23244.08998	84192.20352	8.583984148	9.753431749	0.318539146	11.3170996	1.301927727	0.0312	InVivo/SM>InVivo/FM
tr | F1N1T3 | F1N1T3_BOVIN	Uncharacterized protein OS=Bos taurus GN=AKAP2 PE=4 SV=2	Successfully Matured: In Vitro	20	20	19.579	209664.1214	80746.21701	189210.6567	185126.0907	2.15219831	12.87352723	0.451800469	13.6330698	0.470587889	0.0314	InVivo/FM>InVivo/SM
sp | Q32L22 | RPAC1_BOVIN	DNA-directed RNA polymerases I and III subunit RPAC1 OS=Bos taurus GN=POLR1C PE=2 SV=1	Successfully Matured: In Vitro	3	3	2.954	4043.606967	2168.169755	4385.815308	3978.826065	2.811711258	8.731638189	1.01332024	9.979150913	0.371448387	0.0324	
sp | Q0IE59 | PDXK_BOVIN	Pyridoxal kinase OS=Bos taurus GN=PDXK PE=2 SV=1	Successfully Matured: In Vitro	12	12	11.927	58630.20428	30788.4435	54666.36913	41823.74638	2.237828005	11.51124194	0.711258519	12.42804686	0.369930384	0.0338	InVivo/FM>InVivo/SM; InVivo/FM>InVitro/FM
tr | Q29RM7 | Q29RM7_BOVIN	Fas (TNFRSF6) associated factor 1 OS=Bos taurus GN=FAF1 PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.892	8138.444765	5384.41472	7668.817287	7466.630825	2.246982388	9.503382873	0.714555126	10.4385976	0.417009894	0.0354	InVivo/FM>InVivo/SM
tr | A5D9G1 | A5D9G1_BOVIN	UDP-N-acetylglucosamine pyrophosphorylase 1 OS=Bos taurus GN=UAP1 PE=2 SV=1 [tr | A5D9G1 | A5D9G1_BOVIN,tr | F1MJP7 | F1MJP7_BOVIN]	Successfully Matured: In Vitro	21	20	20.499	170483.3727	114307.0029	190143.9774	194656.0524	2.904040278	12.38329568	1.127287727	13.73462207	0.436986471	0.0370	InVitro/FM>InVivo/FM
tr | F1N3G2 | F1N3G2_BOVIN	Uncharacterized protein OS=Bos taurus GN=NOP2 PE=4 SV=1	Successfully Matured: In Vitro	7	7	6.954	11045.16274	4004.441334	9995.47665	11773.82236	2.165266097	9.93919849	0.421399838	10.67504652	0.506689173	0.0371	
sp | P45478 | PPT1_BOVIN	Palmitoyl-protein thioesterase 1 OS=Bos taurus GN=PPT1 PE=1 SV=1 [sp | P45478 | PPT1_BOVIN,tr | F1MSA1 | F1MSA1_BOVIN]	Successfully Matured: In Vitro	5	5	4.985	189216.7783	81192.2218	169457.6207	188815.4079	2.117280362	12.77405179	0.413663109	13.4982937	0.504792558	0.0380	
sp | P03969 | FGF2_BOVIN	Fibroblast growth factor 2 OS=Bos taurus GN=FGF2 PE=1 SV=1	Successfully Matured: In Vitro	4	4	3.884	21936.48466	10043.02912	19449.24672	20671.43931	2.076993135	10.61730551	0.406495055	11.32751618	0.494440737	0.0380	InVivo/SM>InVivo/FM
tr | Q24K01 | Q24K01_BOVIN;rr | REV_F1ML W0 | REV_F1ML W0_BOVIN;rr | REV_O18960 | REV_O18960_BOVIN;rr | REV_Q0IIB6 | REV_DDT3_BOVIN;rr | REV_Q8HYY3 | REV_Q8HYY3_BOVIN;tr | Q58CW7 | Q58CW7_BOVIN	NudC domain containing 3 OS=Bos taurus GN=NUDCD3 PE=2 SV=1 [tr | Q24K01 | Q24K01_BOVIN,tr | Q58CW7 | Q58CW7_BOVIN]	Successfully Matured: In Vitro	2	2	1.897	896.7220436	1142.791265	1332.866628	3816.991555	4.183752643	7.002145571	1.013435657	8.548381521	0.960620933	0.0383	
tr | F1N1S2 | F1N1S2_BOVIN	Uncharacterized protein OS=Bos taurus GN=MAP1B PE=4 SV=2	Successfully Matured: In Vitro	31	30	30.538	97991.00005	52820.56023	100540.4696	140254.6851	2.590910076	12.09444937	0.448561189	12.98394657	0.671406621	0.0391	InVitro/FM>InVivo/FM
tr | F1N3I3 | F1N3I3_BOVIN	Uncharacterized protein OS=Bos taurus GN=UBE2O PE=4 SV=2	Successfully Matured: In Vitro	2	2	1.904	486.4093939	402.0571604	493.6042895	627.6988521	2.474206643	6.634241339	0.775760639	7.669739441	0.549728002	0.0409	
tr | E1BFP1 | E1BFP1_BOVIN	Uncharacterized protein OS=Bos taurus GN=HEBP2 PE=4 SV=1	Successfully Matured: In Vitro	2	2	1.987	3273.632765	3116.584069	4028.624679	4565.056379	3.282902578	8.151299806	1.493232044	9.877339099	0.541041242	0.0412	InVivo/FM>InVivo/SM
sp | Q29460 | PA1B3_BOVIN	Platelet-activating factor acetylhydrolase IB subunit gamma OS=Bos taurus GN=PAFAH1B3 PE=1 SV=1	Successfully Matured: In Vitro	8	8	7.931	209181.0468	96178.92425	181616.7431	154431.2026	2.007232869	12.82570003	0.597255435	13.58711367	0.36659085	0.0412	
tr | Q08DH4 | Q08DH4_BOVIN	Kinesin light chain 2 OS=Bos taurus GN=KLC2 PE=2 SV=1	Successfully Matured: In Vitro	4	2	3.93	8509.895494	4915.641534	7919.341124	8026.945584	2.219399421	9.591933761	0.629590324	10.45154104	0.49514021	0.0432	
tr | Q1LZ90 | Q1LZ90_BOVIN	Chloride channel, nucleotide-sensitive, 1A OS=Bos taurus GN=CLNS1A PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.957	3315.063645	2843.452464	3835.324161	6842.004266	3.021709832	8.410021518	1.06253438	9.737406651	0.635388098	0.0433	
sp | Q2KHU0 | SERB_BOVIN	Phosphoserine phosphatase OS=Bos taurus GN=PSPH PE=2 SV=1	Successfully Matured: In Vitro	2	2	1.965	7971.428253	6860.551747	9261.411245	8820.943908	3.041408649	9.049562951	1.54085514	10.7339561	0.375333282	0.0449	InVivo/FM>InVivo/SM
tr | F1MVC0 | F1MVC0_BOVIN	Uncharacterized protein OS=Bos taurus GN=CAD PE=3 SV=2	Successfully Matured: In Vitro	8	8	7.914	33089.43364	14722.59595	31669.74569	40943.28538	2.347574581	10.94456815	0.736265524	11.85934898	0.466702814	0.0469	
tr | A6QLY8 | A6QLY8_BOVIN	IGFBP7 protein OS=Bos taurus GN=IGFBP7 PE=2 SV=1 [tr | A6QLY8 | A6QLY8_BOVIN,tr | F1MPP2 | F1MPP2_BOVIN]	Successfully Matured: In Vitro	2	2	1.994	632.161832	746.0549859	1270.85841	1890.090823	6.19338425	4.506295471	4.140366848	8.870664221	0.492877601	0.0474	InVitro/SM>InVitro/FM
tr | A5D7Q4 | A5D7Q4_BOVIN	CSDA protein OS=Bos taurus GN=CSDA PE=2 SV=1	Successfully Matured: In Vitro	8	3	7.925	43771.9657	32327.20452	47180.04196	66775.30431	2.747851883	11.07494909	0.956724227	12.24990296	0.61660819	0.0498	

11.2 *In Vivo*: successfully matured versus failed to mature

						In Vivo: Successfully Matured		In Vivo: Failed to Mature			In Vivo: Successfully Matured		In Vivo: Failed to Mature			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|F1MNW4|F1MNW4_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Bos taurus GN=ITIH2 PE=4 SV=2	In Vivo: Successfully Matured	57	57	55.98	30308712.23	15294296.32	218490.75	46926.14	138.72	17.82	0.45	12.97	0.21	0.0000	InVivo/SM>InVitro/SM
tr|Q5W1C4|Q5W1C4_BOVIN	Tumor necrosis factor, alpha-induced protein 6 OS=Bos taurus GN=tsg-6 PE=2 SV=1	In Vivo: Successfully Matured	15	15	14.80	9645479.45	5390328.89	210495.16	31216.60	45.82	16.65	0.50	12.94	0.15	0.0000	InVivo/SM>InVitro/SM
sp|P46193|ANXA1_BOVIN	Annexin A1 OS=Bos taurus GN=ANXA1 PE=2 SV=2 [sp|P46193|ANXA1_BOVIN,tr|F1N650|F1N650_BOVIN,tr|I6YIV1|I6YIV1_BOVIN]	In Vivo: Successfully Matured	16	16	15.67	541806.11	126874.47	104171.74	18894.87	5.20	13.87	0.21	12.23	0.17	0.0000	InVitro/FM>InVivo/FM
tr|F1MBW3|F1MBW3_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=ACSL4 PE=4 SV=1	In Vivo: Successfully Matured	19	17	18.68	104269.33	20037.23	19768.24	5752.18	5.27	12.23	0.16	10.55	0.29	0.0000	InVitro/SM>InVivo/SM; InVitro/FM>InVivo/FM
tr|G5E513|G5E513_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=1	In Vivo: Successfully Matured	3	2	2.97	40167.89	23517.41	9.57	21.40	4196.94	11.11	0.64	0.91	2.04	0.0000	InVivo/SM>InVitro/SM; InVitroFM>InVivoFM; InVitro/FM>InVitro/SM
sp|O46375|TTHY_BOVIN	Transthyretin OS=Bos taurus GN=TTR PE=1 SV=1	In Vivo: Successfully Matured	5	5	4.93	845318.05	297381.25	62171.93	25076.01	13.60	14.29	0.32	11.66	0.45	0.0000	InVivo/SM>InVitro/SM; InVitro/FM>InVivo/FM
tr|C9EF39|C9EF39_BOVIN;tr|C9HQW1|C9HQW1_BOVIN;tr|K7QE84|K7QE84_BOVIN;tr|O46711|O46711_BOVIN;tr|Q3YJJ6|Q3YJJ6_BOVIN;tr|W5XHU3|W5XHU3_BOVIN	MHC class I antigen (Fragment) OS=Bos taurus GN=BoLA PE=2 SV=1	In Vivo: Successfully Matured	11	4	10.90	8967.10	2908.96	842.70	397.63	10.64	9.75	0.28	7.36	0.41	0.0000	InVitro/SM>InVivo/SM
sp|P81282|CSPG2_BOVIN;tr|F1MZ85|F1MZ85_BOVIN;tr|F1N6I5|F1N6I5_BOVIN	Versican core protein OS=Bos taurus GN=VCAN PE=1 SV=2 [sp|P81282|CSPG2_BOVIN,tr|F1MZ85|F1MZ85_BOVIN]	In Vivo: Successfully Matured	75	12	73.41	388434.22	175515.73	35406.92	7895.93	10.97	13.48	0.40	11.15	0.24	0.0000	InVivo/SM>InVitro/SM
sp|Q2UVX4|CO3_BOVIN	Complement C3 OS=Bos taurus GN=C3 PE=1 SV=2	In Vivo: Successfully Matured	59	59	57.54	4025457.84	1830152.55	202820.72	83490.92	19.85	15.82	0.41	12.83	0.46	0.0000	InVivo/SM>InVitro/SM
sp|P41361|ANT3_BOVIN;zz|ZZ_FGCZcont0237|	Antithrombin-III OS=Bos taurus GN=SERPINC1 PE=1 SV=2	In Vivo: Successfully Matured	15	15	14.89	292834.39	177417.92	13522.44	8924.40	21.66	13.17	0.44	10.08	0.51	0.0000	InVivo/SM>InVitro/SM
sp|P56652|ITIH3_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H3 OS=Bos taurus GN=ITIH3 PE=1 SV=2	In Vivo: Successfully Matured	14	13	13.82	1176688.44	623083.12	17408.33	11988.03	67.59	14.56	0.47	10.21	0.86	0.0000	InVivo/SM>InVitro/SM
tr|F1MMP5|F1MMP5_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Bos taurus GN=ITIH1 PE=4 SV=1	In Vivo: Successfully Matured	94	13	93.11	4168140.03	2888900.46	20782.99	18726.68	200.56	15.69	0.76	10.26	0.98	0.0000	InVivo/SM>InVitro/SM
tr|F1N1I6|F1N1I6_BOVIN;zz|ZZ_FGCZCont0149|	Gelsolin OS=Bos taurus GN=GSN PE=4 SV=1	In Vivo: Successfully Matured	18	17	17.55	1189926.37	169302.09	326485.05	77945.91	3.64	14.67	0.13	13.36	0.28	0.0000	
tr|B0JYQ0|B0JYQ0_BOVIN	ALB protein OS=Bos taurus GN=ALB PE=2 SV=1	In Vivo: Successfully Matured	265	24	260.40	1793789.59	1202116.27	21075.28	7539.08	85.11	14.82	0.82	10.57	0.48	0.0000	InVivo/SM>InVitro/SM
tr|G3N0S9|G3N0S9_BOVIN	Uncharacterized protein OS=Bos taurus GN=LOC515150 PE=4 SV=1	In Vivo: Successfully Matured	10	9	9.87	2986519.61	1818694.53	29234.84	13367.86	102.16	15.36	0.81	10.85	0.61	0.0000	InVivo/SM>InVitro/SM
tr|F1MGU7|F1MGU7_BOVIN	Fibrinogen gamma-B chain OS=Bos taurus GN=FGG PE=4 SV=1 [tr|F1MGU7|F1MGU7_BOVIN,tr|Q3SZZ9|Q3SZZ9_BOVIN]	In Vivo: Successfully Matured	29	29	28.50	5568359.09	2676561.06	113174.18	64168.81	49.20	16.02	0.78	12.23	0.46	0.0000	InVivo/SM>InVitro/SM
sp|P01044|KNG1_BOVIN	Kininogen-1 OS=Bos taurus GN=KNG1 PE=1 SV=1	In Vivo: Successfully Matured	5	5	4.96	87356.31	30506.84	9468.36	2893.88	9.23	12.00	0.40	9.80	0.37	0.0000	InVivo/SM>InVitro/SM
tr|F1MU12|F1MU12_BOVIN;zz|ZZ_FGCZCont0125|	Keratin, type II cytoskeletal 8 OS=Bos taurus GN=KRT8 PE=3 SV=1	In Vivo: Successfully Matured	56	48	55.13	4791317.85	2569515.92	373012.58	177963.90	12.84	15.95	0.50	13.45	0.40	0.0000	InVivo/SM>InVitro/SM
tr|F1MYG5|F1MYG5_BOVIN	Uncharacterized protein OS=Bos taurus GN=LMNA PE=3 SV=1	In Vivo: Successfully Matured	28	28	27.70	1642716.00	493098.44	470290.15	108046.73	3.49	14.97	0.25	13.73	0.22	0.0000	
tr|F1MAV0|F1MAV0_BOVIN	Fibrinogen beta chain OS=Bos taurus GN=FGB PE=4 SV=2	In Vivo: Successfully Matured	32	32	31.39	4718901.49	2389013.97	115601.36	97554.66	40.82	15.86	0.76	12.14	0.66	0.0001	InVivo/SM>InVitro/SM
sp|Q95114|MFGM_BOVIN	Lactadherin OS=Bos taurus GN=MFGE8 PE=1 SV=2 [sp|Q95114|MFGM_BOVIN,tr|F1MXX6|F1MXX6_BOVIN]	In Vivo: Successfully Matured	37	37	36.12	9187928.95	3441874.54	1012203.65	509909.87	9.08	16.67	0.34	14.41	0.54	0.0001	InVitro/FM>InVivo/FM
sp|P02769|ALBU_BOVIN;zz|ZZ_FGCZCont0112|	sp|ALBU_BOVIN|[sp|P02769|ALBU_BOVIN,zz|ZZ_FGCZCont0112|]	In Vivo: Successfully Matured	290	13	284.93	21310501.54	13657548.02	524281.56	348788.29	40.65	17.35	0.72	13.67	0.71	0.0001	InVivo/SM>InVitro/SM
tr|Q3ZBX0|Q3ZBX0_BOVIN	Basigin OS=Bos taurus GN=BSG PE=2 SV=1	In Vivo: Successfully Matured	11	11	10.90	6858620.29	3109190.92	884856.37	195991.21	7.75	16.33	0.47	14.37	0.22	0.0001	InVivo/SM>InVitro/SM
tr|A5PJT7|A5PJT7_BOVIN	ECM1 protein OS=Bos taurus GN=ECM1 PE=2 SV=1	In Vivo: Successfully Matured	3	3	2.99	104661.11	66481.91	3818.54	2347.28	27.41	12.08	0.61	8.77	0.71	0.0001	InVivo/SM>InVitro/SM
sp|P04272|ANXA2_BOVIN;tr|REV_F1MR60|REV_F1MR60_BOVIN;zz|REV_Q29RR5|REV_TFP11_BOVIN	Annexin A2 OS=Bos taurus GN=ANXA2 PE=1 SV=2	In Vivo: Successfully Matured	26	26	25.67	2433205.01	496580.33	620742.74	230006.11	3.92	15.38	0.19	13.98	0.36	0.0001	InVivo/SM>InVitro/SM
sp|P79132|CAV1_BOVIN	Caveolin-1 OS=Bos taurus GN=CAV1 PE=2 SV=1	In Vivo: Successfully Matured	4	4	3.91	75425.68	77215.77	901.91	472.95	83.63	11.43	1.01	7.40	0.48	0.0001	InVivo/SM>InVitro/SM
sp|Q0VC16|MIA3_BOVIN	Melanoma inhibitory activity protein 3 OS=Bos taurus GN=MIA3 PE=2 SV=2 [sp|Q0VC16|MIA3_BOVIN,tr|G5E5L5|G5E5L5_BOVIN]	In Vivo: Successfully Matured	7	7	6.82	231415.02	59094.76	59045.66	16686.71	3.92	13.01	0.26	11.64	0.31	0.0001	InVivo/SM>InVitro/SM
sp|A6QLZ7|CRLD2_BOVIN;tr|D1Z306|D1Z306_BOVIN	Cysteine-rich secretory protein LCCL domain-containing 2 OS=Bos taurus GN=CRISPLD2 PE=2 SV=1 [sp|A6QLZ7|CRLD2_BOVIN,tr|D1Z306|D1Z306_BOVIN]	In Vivo: Successfully Matured	11	10	10.79	251522.33	136471.08	16836.22	13633.57	14.94	13.02	0.47	10.20	0.70	0.0001	InVivo/SM>InVitro/SM
sp|P34955|A1AT_BOVIN	Alpha-1-antiproteinase OS=Bos taurus GN=SERPINA1 PE=1 SV=1	In Vivo: Successfully Matured	12	12	11.66	466765.78	277551.92	32713.07	17576.14	14.27	13.59	0.59	10.99	0.48	0.0001	InVivo/SM>InVitro/SM
sp|P12763|FETUA_BOVIN_trunc	Alpha-2-HS-glycoprotein OS=Bos taurus GN=AHSG PE=1 SV=2	In Vivo: Successfully Matured	7	7	6.88	686449.13	579852.78	22808.24	20658.42	30.10	13.93	0.57	10.41	0.91	0.0001	InVivo/SM>InVitro/SM
tr|Q1RMH5|Q1RMH5_BOVIN	C1QC protein (Fragment) OS=Bos taurus GN=C1QC PE=2 SV=1	In Vivo: Successfully Matured	4	4	3.93	125823.15	83452.47	898.53	659.35	140.03	12.03	1.16	7.24	0.82	0.0001	InVivo/SM>InVitro/SM
sp|Q3SZR3|A1AG_BOVIN	Alpha-1-acid glycoprotein OS=Bos taurus GN=ORM1 PE=2 SV=1 [sp|Q3SZR3|A1AG_BOVIN,tr|Q5GN72|Q5GN72|Q5GN72_BOVIN]	In Vivo: Successfully Matured	7	7	6.87	211655.72	132747.02	6003.89	2855.88	35.25	12.72	0.74	9.23	0.76	0.0001	InVivo/SM>InVitro/SM
tr|A6QPP2|A6QPP2_BOVIN	SERPIND1 protein OS=Bos taurus GN=SERPIND1 PE=2 SV=1	In Vivo: Successfully Matured	3	3	2.98	107819.47	42693.42	7829.67	4966.38	13.77	12.22	0.36	9.43	0.81	0.0001	InVivo/SM>InVitro/SM; InVivo/FM>InVitro/FM
sp|P02672|FIBA_BOVIN	Fibrinogen alpha chain OS=Bos taurus GN=FGA PE=1 SV=5 [sp|P02672|FIBA_BOVIN,tr|A5PJE3|A5PJE3_BOVIN]	In Vivo: Successfully Matured	30	30	29.43	3052108.94	1588131.29	138039.88	43627.67	22.11	15.40	0.80	12.49	0.33	0.0001	InVivo/SM>InVitro/SM
sp|Q3SZV7|HEMO_BOVIN	Hemopexin OS=Bos taurus GN=HPX PE=2 SV=1	In Vivo: Successfully Matured	3	3	2.95	72534.36	31843.66	1667.35	1974.33	43.50	11.80	0.42	7.45	1.34	0.0001	InVivo/SM>InVitro/SM
sp|P00735|THRB_BOVIN	Prothrombin OS=Bos taurus GN=F2 PE=1 SV=2	In Vivo: Successfully Matured	24	24	23.59	778967.71	613170.19	39391.62	11389.58	19.77	13.98	0.76	11.24	0.31	0.0001	InVivo/SM>InVitro/SM
tr|A6H7D3|A6H7D3_BOVIN;tr|F6S1Q0|F6S1Q0_BOVIN;zz|ZZ_FGCZCont0035|;zz|ZZ_FGCZCont0086|	KRT18 protein (Fragment) OS=Bos taurus GN=KRT18 PE=2 SV=1 [tr|A6H7D3|A6H7D3_BOVIN,tr|F6S1Q0|F6S1Q0_BOVIN]	In Vivo: Successfully Matured	24	18	23.41	1700593.63	1167995.95	106530.57	37041.65	15.96	14.82	0.69	12.21	0.39	0.0001	InVivo/SM>InVitro/SM
tr|E1BBS6|E1BBS6_BOVIN	Sulfhydryl oxidase (fragment) OS=Bos taurus GN=QSOX2 PE=3 SV=2	In Vivo: Successfully Matured	6	6	5.86	952252.72	511118.26	75635.63	30176.33	12.59	14.30	0.62	11.86	0.43	0.0002	InVitro/FM>InVivo/FM
sp|A6QPQ2|SPA38_BOVIN	Serpin A3-8 OS=Bos taurus GN=SERPINA3-8 PE=2 SV=1	In Vivo: Successfully Matured	5	2	4.77	28195.92	30099.27	37.30	75.17	755.87	10.36	1.26	2.25	2.38	0.0002	InVivo/SM>InVitro/SM
sp|Q28065|C4BPA_BOVIN	C4b-binding protein alpha chain OS=Bos taurus GN=C4BPA PE=2 SV=1	In Vivo: Successfully Matured	15	10	14.61	182729.93	119276.23	13961.75	2109.05	13.09	12.59	0.71	10.23	0.15	0.0002	InVivo/SM>InVitro/SM
sp|Q28085|CFAH_BOVIN	Complement factor H OS=Bos taurus GN=CFH PE=1 SV=3	In Vivo: Successfully Matured	7	5	6.84	43101.53	37769.71	533.01	182.14	80.86	10.85	1.16	6.91	0.43	0.0002	InVivo/SM>InVitro/SM; InVitro/FM>InVivo/FM
tr|Q8DC0|Q8DC0_BOVIN	Serpin peptidase inhibitor, clade E (Nexin, plasminogen activator inhibitor type 1), member 2 OS=Bos taurus GN=SERPINE2 PE=2 SV=1 [tr|Q8DC0|Q8DC0_BOVIN,tr|Q8HZY1|Q8HZY1_BOVIN]	In Vivo: Successfully Matured	24	24	23.44	4081567.21	1446102.52	522931.46	229968.76	7.81	15.86	0.35	13.74	0.62	0.0002	InVivo/SM>InVitro/SM
sp|P15497|APOA1_BOVIN	Apolipoprotein A-I OS=Bos taurus GN=APOA1 PE=1 SV=3 [sp|P15497|APOA1_BOVIN,tr|V6P9A2|V6P9A2_BOVIN]	In Vivo: Successfully Matured	5	5	4.94	141430.32	69266.53	5570.44	7056.69	25.39	12.48	0.37	8.66	1.27	0.0002	InVivo/SM>InVitro/SM
tr|F1MMK9|F1MMK9_BOVIN	Protein AMBP OS=Bos taurus GN=AMBP PE=4 SV=2	In Vivo: Successfully Matured	7	7	6.82	418683.59	314926.76	9558.56	5706.36	43.80	13.28	0.94	9.67	0.73	0.0002	InVivo/SM>InVitro/SM
sp|P08728|K1C19_BOVIN	Keratin, type I cytoskeletal 19 OS=Bos taurus GN=KRT19 PE=2 SV=1	In Vivo: Successfully Matured	33	17	32.18	1281954.96	545782.68	167732.42	133729.30	7.64	14.69	0.36	12.53	0.65	0.0002	InVivo/SM>InVitro/SM
tr|E1BJK2|E1BJK2_BOVIN	Uncharacterized protein OS=Bos taurus GN=TUBB1 PE=3 SV=1	In Vivo: Successfully Matured	13	1	12.89	6050.40	4425.45	24.51	54.80	246.90	8.95	1.21	1.10	2.46	0.0002	InVivo/SM>InVitro/SM

						In Vivo: Successfully Matured		In Vivo: Failed to Mature			In Vivo: Successfully Matured		In Vivo: Failed to Mature			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|A6QNW7|A6QNW7_BOVIN	CD5L protein OS=Bos taurus GN=CD5L PE=2 SV=1 [tr|A6QNW7|A6QNW7_BOVIN,tr|F1N5U4|F1N5U4_BOVIN]	In Vivo: Successfully Matured	7	7	6.88	45741.45	30077.00	1417.78	700.27	32.26	11.09	0.97	7.85	0.50	0.0003	InVivo/SM>InVitro/SM; InVitro/FM>InVitro/SM; InVitro/FM>InVivo/FM
sp|Q3MHN2|CO9_BOVIN	Complement component C9 OS=Bos taurus GN=C9 PE=2 SV=1	In Vivo: Successfully Matured	6	6	5.89	166034.10	103583.26	15366.85	10521.14	10.80	12.57	0.52	10.14	0.69	0.0003	InVivo/SM>InVitro/SM
tr|F1MY84|F1MY84_BOVIN	Uncharacterized protein OS=Bos taurus GN=CSPG4 PE=4 SV=2	In Vivo: Successfully Matured	30	29	29.76	1036078.48	444562.25	128500.66	75161.35	8.06	14.47	0.37	12.28	0.71	0.0003	InVivo/SM>InVitro/SM
zz|ZZ_FGCZCont0047|	gi|1181994 (X81419) hair type I acidic keratin [Homo sapiens] gi|16687 [zz|ZZ_FGCZCont0047|,zz|ZZ_FGCZCont0144|]	In Vivo: Successfully Matured	7	1	6.77	8161.13	2127.52	238.91	288.81	34.16	9.67	0.23	5.35	1.60	0.0003	InVivo/SM>InVitro/SM
tr|G3X6N3|G3X6N3_BOVIN,sp|P24627|TRFL_BOVIN,tr|B3VTM3|B3VTM3_BOVIN,tr|B9VPZ5|B9VPZ5_BOVIN,tr|C7FE01|C7FE01_BOVIN,tr|E1BH82|E1BH82_BOVIN,tr|Q2HJF0|Q2HJF0_BOVIN,tr|Q6LBN7|Q6LBN7_BOVIN,tr|Q8MII0|Q8MII0_BOVIN	Serotransferrin OS=Bos taurus GN=TF PE=4 SV=1	In Vivo: Successfully Matured	32	31	31.71	2173835.55	1128818.24	157241.26	87743.41	13.82	15.16	0.54	12.45	0.82	0.0003	InVivo/SM>InVitro/SM
tr|A6H741|A6H741_BOVIN	TMEM176B protein OS=Bos taurus GN=TMEM176B PE=2 SV=1	In Vivo: Successfully Matured	3	3	2.94	78277.32	17254.79	5802.64	4559.13	13.49	11.94	0.21	9.01	1.08	0.0004	InVitro/FM>InVivo/FM
tr|F1MD77|F1MD77_BOVIN	Uncharacterized protein OS=Bos taurus GN=LAMC1 PE=4 SV=2	In Vivo: Successfully Matured	26	26	25.60	588606.59	353707.96	45027.83	26179.27	13.07	13.84	0.53	11.20	0.82	0.0004	InVivo/SM>InVitro/SM
sp|Q27966-2|MYO1C_BOVIN	Isoform 2 of Unconventional myosin-Ic OS=Bos taurus GN=MYO1C [sp|Q27966-2|MYO1C_BOVIN,sp|Q27966-3|MYO1C_BOVIN,sp|Q27966|MYO1C_BOVIN]	In Vivo: Successfully Matured	2	2	1.91	1657206.40	1255945.43	51200.28	36246.47	32.37	14.69	0.93	11.31	0.77	0.0004	InVivo/SM>InVitro/SM; InVitro/FM>InVivo/FM
sp|P49907|SEPP1_BOVIN	Selenoprotein P OS=Bos taurus GN=SEPP1 PE=2 SV=2	In Vivo: Successfully Matured	2	2	1.99	8331.97	8789.94	19.67	26.33	423.61	9.07	1.22	2.09	2.32	0.0004	InVivo/SM>InVitro/SM
sp|Q28198|TPA_BOVIN	Tissue-type plasminogen activator OS=Bos taurus GN=PLAT PE=2 SV=1	In Vivo: Successfully Matured	6	6	5.96	117879.35	58178.71	7560.15	5343.81	15.59	12.26	0.48	9.31	1.01	0.0004	InVivo/SM>InVitro/SM
tr|G1K238|G1K238_BOVIN,tr|F1MZU6|F1MZU6_BOVIN,tr|F1N474|F1N474_BOVIN	Collagen alpha-1(IV) chain OS=Bos taurus GN=COL4A1 PE=4 SV=2	In Vivo: Successfully Matured	2	1	1.97	61025.47	43924.97	1908.64	2090.66	31.97	11.49	0.67	7.70	1.27	0.0004	InVivo/SM>InVitro/SM
tr|Q3SZQ8|Q3SZQ8_BOVIN,tr|Q3T0|6|Q3T06_BOVIN,tr|Q5J801|Q5J801_BOVIN	Endopin 2 OS=Bos taurus GN=SERPINA3-7 PE=2 SV=1 [tr|Q3SZQ8|Q3SZQ8_BOVIN,tr|Q5J801|Q5J801_BOVIN]	In Vivo: Successfully Matured	5	5	4.89	223443.42	141325.97	12353.31	8387.49	18.09	12.81	0.68	9.87	0.87	0.0004	InVivo/SM>InVitro/SM
tr|B8Y959|B8Y959_BOVIN	Embryo-specific fibronectin 1 transcript variant OS=Bos taurus GN=FN1 PE=2 SV=1	In Vivo: Successfully Matured	35	35	34.54	1370539.07	586456.93	98374.42	85111.34	13.93	14.76	0.36	11.80	1.08	0.0004	InVivo/SM>InVitro/SM
sp|P06868|PLMN_BOVIN	Plasminogen OS=Bos taurus GN=PLG PE=1 SV=2 [sp|P06868|PLMN_BOVIN,tr|E1BE26|E1BE26_BOVIN]	In Vivo: Successfully Matured	3	3	2.97	25813.50	29530.37	49.87	49.76	517.61	10.45	0.83	3.04	2.79	0.0005	InVivo/SM>InVitro/SM
tr|B0JYP6|B0JYP6_BOVIN	IGK protein OS=Bos taurus GN=IGK PE=2 SV=1 [tr|B0JYP6|B0JYP6_BOVIN,tr|F1M144|F1M144_BOVIN,tr|F1MZ96|F1MZ96_BOVIN,tr|Q05B55|Q05B55_BOVIN]	In Vivo: Successfully Matured	7	6	6.94	222831.48	190342.53	7736.28	4047.37	28.80	12.65	0.92	9.47	0.76	0.0005	InVivo/SM>InVitro/SM
tr|Q9TS85|Q9TS85_BOVIN,tr|Q9BGU1|Q9BGU1_BOVIN	Histidine-rich GLYCOPROTEIN--FACTOR XIIIA substrate (Fragments) OS=Bos taurus PE=1 SV=1	In Vivo: Successfully Matured	3	1	2.99	37621.95	19031.90	132.85	233.78	283.18	11.13	0.45	2.50	3.46	0.0006	InVivo/SM>InVitro/SM
tr|G3MX98|G3MX98_BOVIN	Uncharacterized protein OS=Bos taurus GN=KRT9 PE=4 SV=1	In Vivo: Successfully Matured	3	1	2.79	247666.82	201607.92	8383.26	8947.61	29.54	12.77	0.90	9.31	0.99	0.0006	InVivo/SM>InVitro/SM; InVitro/FM>InVivo/FM
sp|Q29RQ1|CO7_BOVIN	Complement component C7 OS=Bos taurus GN=C7 PE=2 SV=1 [sp|Q29RQ1|CO7_BOVIN,tr|F1N045|F1N045_BOVIN]	In Vivo: Successfully Matured	2	2	1.89	4074.15	5253.25	173.26	110.06	23.51	8.51	0.90	5.70	0.59	0.0006	InVivo/SM>InVitro/SM
sp|P80724|BASP1_BOVIN	Brain acid soluble protein 1 OS=Bos taurus GN=BASP1 PE=1 SV=3	In Vivo: Successfully Matured	2	2	1.98	1809.03	2416.17	7.23	9.81	250.33	7.39	1.38	1.49	1.90	0.0007	InVitro/FM>InVivo/FM
tr|E1BPK6|E1BPK6_BOVIN	Uncharacterized protein OS=Bos taurus GN=MYO6 PE=4 SV=2	In Vivo: Successfully Matured	18	18	17.49	248960.55	38870.32	95851.26	31559.16	2.60	13.11	0.15	12.11	0.38	0.0007	InVitro/FM>InVivo/FM
tr|F1MCK2|F1MCK2_BOVIN	Uncharacterized protein OS=Bos taurus GN=AHNAK PE=4 SV=2	In Vivo: Successfully Matured	7	7	6.85	167820.30	53360.08	41207.46	21787.14	4.07	12.69	0.27	11.20	0.55	0.0007	InVitro/FM>InVivo/FM
sp|O18824|SCRB1_BOVIN	Scavenger receptor class B member 1 OS=Bos taurus GN=SCARB1 PE=2 SV=1 [sp|O18824|SCRB1_BOVIN,tr|A4IFC6|A4IFC6_BOVIN]	In Vivo: Successfully Matured	2	2	1.94	67982.05	50875.11	4371.99	2674.04	15.55	11.56	0.77	8.88	0.78	0.0008	InVivo/SM>InVitro/SM
tr|E1BGF8|E1BGF8_BOVIN	Uncharacterized protein OS=Bos taurus GN=AGPAT9 PE=4 SV=1	In Vivo: Successfully Matured	6	6	5.95	71550.75	15421.26	8042.43	5357.60	8.90	11.85	0.19	9.36	1.06	0.0009	InVitro/FM>InVivo/FM
sp|P19660|CTHL2_BOVIN	Cathelicidin-2 OS=Bos taurus GN=CATHL2 PE=1 SV=2	In Vivo: Successfully Matured	2	2	1.99	12295.52	10673.19	54.07	53.39	227.42	9.64	1.11	3.48	2.38	0.0009	InVivo/SM>InVitro/SM
sp|A2I7N1|SPA35_BOVIN	Serpin A3-5 OS=Bos taurus GN=SERPINA3-5 PE=3 SV=1 [sp|A2I7N1|SPA35_BOVIN,tr|G8JKW7|G8JKW7_BOVIN]	In Vivo: Successfully Matured	9	1	8.79	51173.21	33922.39	2670.53	3914.36	19.16	11.38	0.56	7.71	1.48	0.0009	InVivo/SM>InVitro/SM
sp|Q3MHN5|VTDB_BOVIN	Vitamin D-binding protein OS=Bos taurus GN=GC PE=2 SV=1 [sp|Q3MHN5|VTDB_BOVIN,tr|F1N5M2|F1N5M2_BOVIN,tr|I7CT57|I7CT57_BOVIN]	In Vivo: Successfully Matured	5	5	4.91	53455.78	34872.24	2638.86	866.46	20.26	11.23	1.02	8.53	0.35	0.0009	InVivo/SM>InVitro/SM
tr|Q2KII3|Q2KII3_BOVIN	Hepatitis A virus cellular receptor 1 N-terminal domain containing protein OS=Bos taurus GN=MGC137099 PE=2 SV=1	In Vivo: Successfully Matured	2	2	1.97	121783.39	83112.94	2246.91	2459.67	54.20	12.01	1.08	7.69	1.48	0.0010	InVivo/SM>InVitro/SM
tr|Q3T101|Q3T101_BOVIN	IGL@ protein OS=Bos taurus GN=IGL@ PE=1 SV=1	In Vivo: Successfully Matured	16	1	15.77	186842.77	63862.64	22665.85	17421.33	8.24	12.77	0.38	10.42	0.95	0.0010	InVivo/SM>InVitro/SM
tr|F1MYN5|F1MYN5_BOVIN	Uncharacterized protein OS=Bos taurus GN=FBLN1 PE=4 SV=2	In Vivo: Successfully Matured	19	19	18.76	4916292.37	2019309.44	630320.49	401332.39	7.80	16.04	0.35	13.80	0.91	0.0010	InVivo/SM>InVitro/SM; InVivo/FM>InVitro/FM
tr|E1BH06|E1BH06_BOVIN	Uncharacterized protein OS=Bos taurus GN=C4A PE=4 SV=2	In Vivo: Successfully Matured	18	3	17.83	74256.56	31383.54	2758.57	3093.29	26.92	11.82	0.44	7.71	1.76	0.0010	InVivo/SM>InVitro/SM
zz|ZZ_FGCZCont0243|	gi|2497269|sp|Q99456|K1C1_HUMAN KERATIN, TYPE I CYTOSKELETAL 12 (CYTO	In Vivo: Successfully Matured	11	1	10.72	36499.75	22538.34	2130.05	1798.71	17.14	10.92	0.89	8.10	0.77	0.0011	InVivo/SM>InVitro/SM
tr|Q3ZBS7|Q3ZBS7_BOVIN	Uncharacterized protein OS=Bos taurus GN=VTN PE=2 SV=1	In Vivo: Successfully Matured	4	4	3.87	563087.12	449881.08	33553.45	35632.07	16.78	13.68	0.73	10.63	1.11	0.0011	InVivo/SM>InVitro/SM
tr|F1MLW8|F1MLW8_BOVIN	Uncharacterized protein OS=Bos taurus PE=4 SV=2	In Vivo: Successfully Matured	16	3	15.78	71204.71	40233.95	2980.23	3529.68	23.89	11.67	0.71	7.85	1.54	0.0011	InVivo/SM>InVitro/SM
sp|P01966|HBA_BOVIN	Hemoglobin subunit alpha OS=Bos taurus GN=HBA PE=1 SV=2	In Vivo: Successfully Matured	15	15	14.61	16663176.62	11973473.07	634706.72	1108641.21	26.25	16.97	0.98	13.08	1.38	0.0011	InVivo/SM>InVitro/SM
tr|F1MTY9|F1MTY9_BOVIN	Heme oxygenase (Decycling) 2 OS=Bos taurus GN=HMOX2 PE=4 SV=2	In Vivo: Successfully Matured	7	7	6.89	231333.83	138268.64	48941.48	17451.51	4.73	12.91	0.50	11.44	0.36	0.0012	InVivo/SM>InVitro/SM
tr|A4IFB8|A4IFB8_BOVIN	IPO4 protein OS=Bos taurus GN=IPO4 PE=2 SV=1	In Vivo: Successfully Matured	4	4	3.95	311565.64	180020.13	19208.31	11995.96	16.22	13.07	0.89	10.37	0.73	0.0012	InVivo/SM>InVitro/SM
sp|Q2KJF1|A1BG_BOVIN	Alpha-1B-glycoprotein OS=Bos taurus GN=A1BG PE=1 SV=1	In Vivo: Successfully Matured	5	5	4.88	55873.50	38002.57	2892.44	2442.00	19.32	11.39	0.74	8.20	1.22	0.0013	InVivo/SM>InVitro/SM
tr|G3N0V0|G3N0V0_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=1	In Vivo: Successfully Matured	11	11	10.78	3149870.65	1989594.86	211027.67	160830.71	14.93	15.40	0.84	12.69	0.87	0.0015	InVivo/SM>InVitro/SM
sp|Q28042|OVGP1_BOVIN	Oviduct-specific glycoprotein (Fragment) OS=Bos taurus GN=OVGP1 PE=1 SV=1 [sp|Q28042|OVGP1_BOVIN,tr|A1L579|A1L579_BOVIN]	In Vivo: Successfully Matured	2	2	1.91	11127.47	16124.89	32.76	30.43	339.62	8.86	1.98	3.77	1.07	0.0017	InVivo/SM>InVitro/SM
tr|Q1RMN9|Q1RMN9_BOVIN	C4b-binding protein alpha-like OS=Bos taurus GN=LOC510860 PE=2 SV=1	In Vivo: Successfully Matured	6	1	5.87	2398.77	2006.11	40.00	56.84	59.96	8.17	0.78	2.75	2.50	0.0018	InVivo/SM>InVitro/SM
sp|Q3T052|ITIH4_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Bos taurus GN=ITIH4 PE=1 SV=1 [sp|Q3T052|ITIH4_BOVIN,tr|F1MMD7|F1MMD7_BOVIN,tr|Q5EA67|Q5EA67_BOVIN]	In Vivo: Successfully Matured	5	5	4.86	94252.69	52470.39	4666.64	6478.01	20.20	12.04	0.45	7.84	2.03	0.0020	InVivo/SM>InVitro/SM
sp|Q7SIH1|A2MG_BOVIN	Alpha-2-macroglobulin OS=Bos taurus GN=A2M PE=1 SV=2	In Vivo: Successfully Matured	16	5	15.70	126278.38	91994.17	8295.97	14273.39	15.22	12.16	0.79	8.70	1.49	0.0021	InVivo/SM>InVitro/SM
tr|A5PKH0|A5PKH0_BOVIN	TOR1AIP2 protein OS=Bos taurus GN=TOR1AIP2 PE=2 SV=1	In Vivo: Successfully Matured	3	3	2.96	49726.47	27090.99	3687.69	2208.59	13.48	11.38	0.51	8.50	1.34	0.0022	InVivo/SM>InVitro/SM

						In Vivo: Successfully Matured		In Vivo: Failed to Mature			In Vivo: Successfully Matured		In Vivo: Failed to Mature			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
sp|Q29437|AOCX_BOVIN	Primary amine oxidase, liver isozyme OS=Bos taurus PE=1 SV=1	In Vivo: Successfully Matured	2	2	1.96	8912.17	5242.32	234.68	367.84	37.98	9.59	0.71	4.61	2.39	0.0022	InVivo/SM>InVitro/SM; InVivo/FM>InVitro/FM; InVitro/SM>InVitro/FM
tr|A6QNM9|A6QNM9_BOVIN	SLC25A12 protein OS=Bos taurus GN=SLC25A12 PE=2 SV=1	In Vivo: Successfully Matured	7	5	6.92	42966.88	26044.44	3117.84	1974.40	13.78	11.22	0.52	8.31	1.35	0.0022	InVivo/SM>InVitro/SM
tr|G3X771|G3X771_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=ABCB1 PE=3 SV=1	In Vivo: Successfully Matured	8	6	7.82	45603.88	48126.92	1824.55	1434.29	24.99	10.91	1.05	7.87	1.01	0.0023	InVitro/FM>InVivo/FM
tr|A6QM09|A6QM09_BOVIN	Putative uncharacterized protein OS=Bos taurus PE=2 SV=1	In Vivo: Successfully Matured	17	2	16.69	32960.08	29759.85	2025.48	890.37	16.27	10.65	1.02	8.21	0.50	0.0024	InVivo/SM>InVitro/SM
tr|F1MEG3|F1MEG3_BOVIN	Uncharacterized protein OS=Bos taurus GN=LAMA1 PE=4 SV=2	In Vivo: Successfully Matured	42	42	41.15	714720.60	536740.68	107802.70	48045.10	6.63	13.94	0.68	12.19	0.49	0.0026	InVivo/SM>InVitro/SM
tr|F1N3L7|F1N3L7_BOVIN;tr|E1BK77|E1BK77_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=DOCK7 PE=4 SV=2	In Vivo: Successfully Matured	7	7	6.87	18923.71	3350.44	5461.06	3227.92	3.47	10.53	0.16	9.12	0.72	0.0027	
tr|D1Z308|D1Z308_BOVIN;tr|G5E6M0|G5E6M0_BOVIN;tr|I9QD97|I9QD97_BOVIN;tr|I9QDG2|I9QDG2_BOVIN	Periostin OS=Bos taurus GN=POSTN PE=2 SV=1 [tr|D1Z308|D1Z308_BOVIN,tr|G5E6M0|G5E6M0_BOVIN,tr|I9QD97|I9QD97_BOVIN,tr|I9QDG2|I9QDG2_BOVIN]	In Vivo: Successfully Matured	8	1	7.95	6912.10	7192.31	59.97	92.90	115.25	8.87	1.45	2.60	2.85	0.0027	InVivo/SM>InVitro/SM
sp|P23805|CONG_BOVIN;sp|P42916|CL43_BOVIN;tr|B7FEK7|B7FEK7_BOVIN;tr|F1MFY6|F1MFY6_BOVIN	Conglutinin OS=Bos taurus GN=CGN1 PE=1 SV=2	In Vivo: Successfully Matured	10	10	9.81	309791.36	198881.93	2949.40	1248.16	105.04	12.61	1.84	8.58	0.56	0.0029	InVivo/SM>InVitro/SM
tr|F1N1W3|F1N1W3_BOVIN	Thrombospondin-2 OS=Bos taurus GN=THBS2 PE=4 SV=1	In Vivo: Successfully Matured	9	8	8.94	16461.56	11322.11	1964.34	799.61	8.38	10.13	0.82	8.19	0.48	0.0030	InVitro/FM>InVivo/FM
sp|Q9TUM6|PLIN2_BOVIN	Perilipin-2 OS=Bos taurus GN=PLIN2 PE=2 SV=1 [sp|Q9TUM6|PLIN2_BOVIN,tr|A1L5C2|A1L5C2_BOVIN]	In Vivo: Successfully Matured	7	7	6.91	90984.43	50201.21	10627.57	11163.96	8.56	12.00	0.47	9.37	1.30	0.0030	
tr|E1BFW2|E1BFW2_BOVIN	Uncharacterized protein OS=Bos taurus GN=IWS1 PE=4 SV=2	In Vivo: Successfully Matured	2	1	1.84	119954.37	77791.56	3562.25	4778.16	33.67	11.96	1.18	8.00	1.66	0.0031	
sp|P30932|CD9_BOVIN	CD9 antigen OS=Bos taurus GN=CD9 PE=2 SV=2 [sp|P30932|CD9_BOVIN,tr|G8JKX6|G8JKX6_BOVIN]	In Vivo: Successfully Matured	4	3	3.87	68888.35	41970.95	11772.29	5217.15	5.85	11.64	0.66	9.97	0.51	0.0031	InVitro/FM>InVivo/FM
tr|Q32LF7|Q32LF7_BOVIN	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 4 OS=Bos taurus GN=B4GALT4 PE=2 SV=1	In Vivo: Successfully Matured	9	9	8.77	369396.80	59102.39	101006.76	48142.40	3.66	13.50	0.14	12.05	0.77	0.0031	InVivo/SM>InVitro/SM
sp|Q9N2I2|IPSP_BOVIN	Plasma serine protease inhibitor OS=Bos taurus GN=SERPINA5 PE=1 SV=1	In Vivo: Successfully Matured	2	2	1.93	4233.34	5786.14	41.48	72.58	102.07	8.04	1.50	3.07	2.08	0.0032	InVivo/SM>InVitro/SM
tr|G3MWS9|G3MWS9_BOVIN	Uncharacterized protein OS=Bos taurus PE=4 SV=1	In Vivo: Successfully Matured	2	2	1.98	5446.65	5332.01	60.73	114.32	89.68	8.53	1.62	2.13	2.99	0.0035	
tr|E1BB91|E1BB91_BOVIN	Uncharacterized protein OS=Bos taurus GN=COL6A3 PE=4 SV=1	In Vivo: Successfully Matured	2	2	1.88	1512.31	2090.11	0.10	0.23	14882.94	6.13	2.95	0.10	0.22	0.0035	InVivo/SM>InVitro/SM
sp|P02453|CO1A1_BOVIN	Collagen alpha-1(I) chain OS=Bos taurus GN=COL1A1 PE=1 SV=3	In Vivo: Successfully Matured	3	3	2.99	93616.34	47853.31	9577.61	7052.43	9.77	12.00	0.58	9.46	1.24	0.0036	InVivo/SM>InVitro/SM
sp|Q5E9X4|LRC59_BOVIN	Leucine-rich repeat-containing protein 59 OS=Bos taurus GN=LRRC59 PE=2 SV=1	In Vivo: Successfully Matured	14	14	13.91	449303.36	122959.61	161690.49	73595.28	2.78	13.68	0.26	12.59	0.53	0.0037	
tr|A6QQ25|A6QQ25_BOVIN	Lysosomal protein transmembrane 4 beta OS=Bos taurus GN=LAPTM4B PE=2 SV=1	In Vivo: Successfully Matured	2	2	1.99	73282.22	32240.03	7381.43	8777.74	9.93	11.80	0.47	8.83	1.59	0.0041	InVitro/FM>InVivo/FM
tr|F1MX87|F1MX87_BOVIN	Uncharacterized protein OS=Bos taurus GN=C8A PE=4 SV=1 [tr|F1MX87|F1MX87_BOVIN,tr|Q1JPD0|Q1JPD0_BOVIN,tr|Q2KIH5|Q2KIH5_BOVIN]	In Vivo: Successfully Matured	2	2	1.99	21021.65	21073.30	259.47	284.37	81.02	10.25	0.88	4.84	2.88	0.0041	InVivo/SM>InVitro/SM
sp|Q7YQJ4|T176A_BOVIN	Transmembrane protein 176A OS=Bos taurus GN=TMEM176A PE=2 SV=1	In Vivo: Successfully Matured	4	4	3.90	16903.20	9791.28	2300.28	2207.50	7.35	10.22	0.72	8.12	0.87	0.0042	InVitro/FM>InVivo/FM
sp|P80747|ITB5_BOVIN	Integrin beta-5 OS=Bos taurus GN=ITGB5 PE=1 SV=2	In Vivo: Successfully Matured	7	7	6.88	109286.43	123851.08	3058.74	2604.81	35.73	11.51	1.39	8.49	0.71	0.0042	InVivo/SM>InVitro/SM
sp|Q2TBX5|SSRD_BOVIN	Translocon-associated protein subunit delta OS=Bos taurus GN=SSR4 PE=2 SV=1	In Vivo: Successfully Matured	6	6	5.98	258931.48	50309.53	114776.51	42785.54	2.26	13.14	0.18	12.27	0.45	0.0046	InVivo/SM>InVitro/SM
tr|A6QR14|A6QR14_BOVIN	DHCR24 protein OS=Bos taurus GN=DHCR24 PE=2 SV=1	In Vivo: Successfully Matured	11	11	10.82	726995.51	331395.36	149050.45	117046.08	4.88	14.12	0.38	12.28	0.97	0.0046	InVivo/SM>InVitro/SM
sp|P03969|FGF2_BOVIN	Fibroblast growth factor 2 OS=Bos taurus GN=FGF2 PE=1 SV=1	In Vivo: Successfully Matured	4	4	3.88	21936.48	10043.03	2328.75	2948.84	9.42	10.62	0.36	7.48	1.76	0.0047	InVitro/SM>InVivo/SM
tr|F1MYG0|F1MYG0_BOVIN	Ornithine aminotransferase, mitochondrial OS=Bos taurus GN=OAT PE=3 SV=1	In Vivo: Successfully Matured	47	47	46.44	10684822.71	5810641.04	2994259.99	1219383.73	3.57	16.76	0.48	15.53	0.46	0.0048	InVivo/SM>InVitro/SM
tr|Q08DL0|Q08DL0_BOVIN	SLC3A2 protein OS=Bos taurus GN=SLC3A2 PE=2 SV=1 [tr|Q08DL0|Q08DL0_BOVIN,tr|Q3T0F0|Q3T0F0_BOVIN,tr|Q5EA54|Q5EA54_BOVIN]	In Vivo: Successfully Matured	4	4	3.93	37535.48	13282.33	9329.60	5633.92	4.02	11.18	0.31	9.61	0.84	0.0049	InVivo/SM>InVitro/SM
tr|Q17QL5|Q17QL5_BOVIN	Transmembrane protein 30A OS=Bos taurus GN=TMEM30A PE=2 SV=1	In Vivo: Successfully Matured	9	9	8.79	87195.74	65657.56	14249.46	10183.44	6.12	11.88	0.58	9.99	0.89	0.0051	InVivo/SM>InVitro/SM
tr|F1ME65|F1ME65_BOVIN	Uncharacterized protein OS=Bos taurus GN=CKAP4 PE=4 SV=1	In Vivo: Successfully Matured	47	47	46.12	11684134.87	2815242.47	4805022.08	1837682.44	2.43	16.94	0.21	15.99	0.50	0.0052	InVivo/SM>InVitro/SM
tr|F1MLG1|F1MLG1_BOVIN	Uncharacterized protein OS=Bos taurus GN=SRPRB PE=4 SV=2	In Vivo: Successfully Matured	4	4	3.98	214837.76	61968.75	92898.88	34887.89	2.31	12.94	0.24	12.06	0.45	0.0055	
tr|F1N7Q7|F1N7Q7_BOVIN	Collagen alpha-2(IV) chain (Fragment) OS=Bos taurus GN=COL4A2 PE=4 SV=2	In Vivo: Successfully Matured	4	4	3.75	74493.47	78966.92	3932.64	1979.04	18.94	11.32	1.11	8.76	0.87	0.0055	InVivo/SM>InVitro/SM
tr|G3N2D7|G3N2D7_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=IGLL1 PE=4 SV=1	In Vivo: Successfully Matured	2	2	1.90	680116.77	279136.96	48249.05	45598.18	14.10	14.04	0.41	10.63	1.98	0.0056	InVivo/SM>InVitro/SM
sp|Q5E9M1|PRAF3_BOVIN	PRA1 family protein 3 OS=Bos taurus GN=ARL6IP5 PE=2 SV=1	In Vivo: Successfully Matured	9	9	8.85	383323.89	120917.08	156677.48	58331.29	2.45	13.51	0.27	12.58	0.47	0.0059	InVivo/SM>InVitro/SM
sp|Q0VCG9|PTX3_BOVIN	Pentraxin-related protein PTX3 OS=Bos taurus GN=PTX3 PE=2 SV=1	In Vivo: Successfully Matured	11	11	10.81	475571.23	558541.69	16320.06	6872.07	29.14	12.95	1.35	10.29	0.57	0.0061	InVivo/SM>InVitro/SM
sp|P67810|SC11A_BOVIN;tr|Q9BDH8|Q9BDH8_BOVIN	Signal peptidase complex catalytic subunit SEC11A OS=Bos taurus GN=SEC11A PE=2 SV=1	In Vivo: Successfully Matured	5	5	4.84	161226.50	34748.36	78565.70	29502.53	2.05	12.67	0.18	11.90	0.44	0.0074	InVivo/SM>InVitro/SM
sp|P21398|AOFA_BOVIN	Amine oxidase [flavin-containing] A OS=Bos taurus GN=MAOA PE=2 SV=2	In Vivo: Successfully Matured	13	13	12.66	549854.78	332042.74	158880.02	61488.06	3.46	13.77	0.53	12.60	0.45	0.0077	InVivo/SM>InVitro/SM
tr|G3MZ03|G3MZ03_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=A4GALT PE=4 SV=1	In Vivo: Successfully Matured	2	2	1.96	3393.52	2961.73	53.50	52.06	63.42	8.04	1.60	3.60	2.20	0.0080	InVivo/SM>InVitro/SM
tr|Q08DM3|Q08DM3_BOVIN	Malic enzyme OS=Bos taurus GN=ME2 PE=2 SV=1	In Vivo: Successfully Matured	8	8	7.77	207931.18	104032.40	71761.68	30773.22	2.90	12.84	0.44	11.79	0.47	0.0089	
sp|Q5E9F5|TAGL2_BOVIN;sp|Q3ZBY2|TAGL3_BOVIN	Transgelin-2 OS=Bos taurus GN=TAGLN2 PE=2 SV=3	In Vivo: Successfully Matured	15	15	14.89	558215.73	247865.66	211461.58	76547.88	2.64	13.84	0.43	12.90	0.39	0.0091	InVitro/FM>InVivo/FM
tr|E1BDK6|E1BDK6_BOVIN;tr|REV_E1BJ09|REV_E1BJ09_BOVIN	Uncharacterized protein OS=Bos taurus GN=LAMB2 PE=4 SV=2	In Vivo: Successfully Matured	25	25	24.59	564777.98	444938.66	84432.81	59377.50	6.69	13.67	0.73	11.73	0.99	0.0095	InVivo/SM>InVitro/SM
sp|Q28156|PDE5A_BOVIN	cGMP-specific 3',5'-cyclic phosphodiesterase OS=Bos taurus GN=PDE5A PE=1 SV=1	In Vivo: Successfully Matured	10	10	9.70	56075.15	44635.53	2446.18	2418.92	22.92	11.00	1.44	8.04	1.12	0.0096	InVitro/FM>InVivo/FM
tr|A6H732|A6H732_BOVIN	TMX4 protein OS=Bos taurus GN=TMX4 PE=2 SV=1	In Vivo: Successfully Matured	5	5	4.99	163135.00	32953.12	80816.65	30957.46	2.02	12.68	0.18	11.92	0.47	0.0098	
sp|Q9XSK2|CD63_BOVIN	CD63 antigen OS=Bos taurus GN=CD63 PE=2 SV=4 [sp|Q9XSK2|CD63_BOVIN,tr|B0JYM4|B0JYM4_BOVIN]	In Vivo: Successfully Matured	4	4	3.69	53863.28	26071.18	24335.66	4554.33	2.21	11.50	0.39	10.78	0.20	0.0099	InVitro/FM>InVivo/FM
tr|E1BP48|E1BP48_BOVIN	Uncharacterized protein OS=Bos taurus GN=KHDRBS2 PE=4 SV=2	In Vivo: Successfully Matured	2	1	1.90	21345.17	31498.72	480.57	290.22	44.42	9.60	1.62	6.73	0.58	0.0099	InVivo/SM>InVitro/SM; InVitro/FM>InVitro/SM
tr|D4QBB3|D4QBB3_BOVIN	Hemoglobin beta OS=Bos taurus GN=HBB PE=3 SV=1	In Vivo: Successfully Matured	26	2	25.49	1057453.17	1203632.89	24850.30	29454.27	42.55	13.63	1.74	10.42	0.89	0.0100	InVivo/SM>InVitro/SM; InVivo/FM>InVitro/FM
sp|Q2NL17|CLPT1_BOVIN	Cleft lip and palate transmembrane protein 1 homolog OS=Bos taurus GN=CLPTM1 PE=2 SV=1	In Vivo: Successfully Matured	6	6	5.96	33116.27	22577.74	6304.61	4132.27	5.25	10.88	0.70	9.23	0.78	0.0101	InVivo/SM>InVitro/SM
sp|Q5EA40|BCAT2_BOVIN	Branched-chain-amino-acid aminotransferase, mitochondrial OS=Bos taurus GN=BCAT2 PE=2 SV=1 [sp|Q5EA40|BCAT2_BOVIN,tr|Q0V8J6|Q0V8J6_BOVIN]	In Vivo: Successfully Matured	4	4	3.95	251817.43	293710.92	9263.25	4936.79	27.18	12.25	1.45	9.72	0.52	0.0104	InVivo/SM>InVitro/SM

						In Vivo: Successfully Matured		In Vivo: Failed to Mature			In Vivo: Successfully Matured		In Vivo: Failed to Mature			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|F1MPR3|F1MPR3_BOVIN;tr|F1MG E7|F1MGE7_BOVIN	Uncharacterized protein OS=Bos taurus GN=ATP2A2 PE=3 SV=2	In Vivo: Successfully Matured	36	30	35.38	930163.72	236518.04	361105.58	156445.81	2.58	14.41	0.21	13.36	0.67	0.0104	InVivo/SM>InVitro/SM
sp|Q05204|LAMP1_BOVIN	Lysosome-associated membrane glycoprotein 1 OS=Bos taurus GN=LAMP1 PE=1 SV=2	In Vivo: Successfully Matured	3	3	2.89	71043.72	37754.16	26277.10	6717.14	2.70	11.75	0.49	10.84	0.28	0.0109	
sp|P02070|HBB_BOVIN;tr|D4QBB4|D4QBB4_BOVIN;zz|ZZ_FGCZContI17 4|	Hemoglobin subunit beta OS=Bos taurus GN=HBB PE=1 SV=1 [sp|P02070|HBB_BOVIN,tr|D4QBB 4|D4QBB4_BOVIN]	In Vivo: Successfully Matured	30	6	29.39	2076138.14	1672666.35	367720.03	434176.47	5.65	14.95	0.80	13.12	0.87	0.0111	InVivo/SM>InVitro/SM
tr|F1MVK1|F1MVK1_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=2	In Vivo: Successfully Matured	11	1	10.89	7692.51	6207.83	368.86	458.71	20.85	9.00	1.47	6.02	1.22	0.0117	InVivo/SM>InVitro/SM
tr|E1BGU2|E1BGU2_BOVIN;tr|E1BQ I5|E1BQ15_BOVIN;tr|G3N2Y5|G3N2Y 5_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=CLASP1 PE=4 SV=2 [tr|E1BGU2|E1BGU2_BOVIN,tr|G3 N2Y5|G3N2Y5_BOVIN]	In Vivo: Successfully Matured	10	10	9.85	57881.30	23622.65	16987.16	9354.62	3.41	11.59	0.38	10.22	0.84	0.0118	
sp|P08169|MPRI_BOVIN	Cation-independent mannose-6-phosphate receptor OS=Bos taurus GN=IGF2R PE=1 SV=2	In Vivo: Successfully Matured	13	13	12.56	154429.55	77383.77	58431.57	30254.07	2.64	12.55	0.41	11.56	0.51	0.0119	
tr|E1BN05|E1BN05_BOVIN	Uncharacterized protein OS=Bos taurus GN=GLDN PE=4 SV=2	In Vivo: Successfully Matured	3	3	2.98	5619.32	6697.64	210.18	254.51	26.74	8.64	1.31	4.78	2.23	0.0121	InVivo/SM>InVitro/SM
sp|A6QLP7|SSRA_BOVIN	Translocon-associated protein subunit alpha OS=Bos taurus GN=SSR1 PE=2 SV=1	In Vivo: Successfully Matured	3	3	2.98	253579.29	36443.74	119434.85	56395.80	2.12	13.13	0.13	12.27	0.58	0.0122	
sp|Q0VCM5|ITIH1_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Bos taurus GN=ITIH1 PE=2 SV=1	In Vivo: Successfully Matured	88	7	87.15	695937.29	826212.56	6935.60	3974.05	100.34	12.94	2.13	9.34	0.79	0.0124	InVivo/SM>InVitro/SM
sp|Q0P5J4|K1C25_BOVIN	Keratin, type I cytoskeletal 25 OS=Bos taurus GN=KRT25 PE=2 SV=1	In Vivo: Successfully Matured	11	1	10.95	6254.52	4279.12	1103.67	977.02	5.67	9.17	0.78	7.38	0.90	0.0125	InVitro/FM>InVivo/FM
sp|Q3T0N3|TMCO1_BOVIN	Transmembrane and coiled-coil domain-containing protein 1 OS=Bos taurus GN=TMCO1 PE=2 SV=1	In Vivo: Successfully Matured	3	3	2.91	17458.00	3555.35	8058.97	3642.14	2.17	10.44	0.18	9.58	0.57	0.0128	InVivo/SM>InVitro/SM
tr|A6QP98|A6QP98_BOVIN	LOC529366 protein (Fragment) OS=Bos taurus GN=LOC529366 PE=2 SV=1 [tr|A6QP98|A6QP98_BOVIN,tr|E1 BMC6|E1BMC6_BOVIN]	In Vivo: Successfully Matured	8	8	7.81	120618.76	103855.50	12634.25	7418.76	9.55	12.00	0.96	9.83	1.10	0.0133	InVivo/SM>InVitro/SM
sp|Q3MHG1|SPTC1_BOVIN	Serine palmitoyltransferase 1 OS=Bos taurus GN=SPTLC1 PE=2 SV=1	In Vivo: Successfully Matured	2	2	1.99	24417.31	11027.84	4790.00	6538.11	5.10	10.71	0.41	8.22	1.71	0.0136	InVivo/SM>InVitro/SM
tr|F1MYW7|F1MYW7_BOVIN	Uncharacterized protein OS=Bos taurus GN=HM13 PE=4 SV=1	In Vivo: Successfully Matured	4	4	3.97	60971.50	25734.11	25934.24	11194.20	2.35	11.65	0.34	10.77	0.50	0.0137	InVivo/SM>InVitro/SM
tr|A3KMW5|A3KMW5_BOVIN	C1QTNF1 protein OS=Bos taurus GN=C1QTNF1 PE=2 SV=1	In Vivo: Successfully Matured	4	4	3.87	56338.43	45915.57	9250.38	5368.96	6.09	11.38	0.69	9.53	1.07	0.0138	InVivo/SM>InVitro/SM
sp|Q3SYT6|CLGN_BOVIN	Calmegin OS=Bos taurus GN=CLGN PE=2 SV=1 [sp|Q3SYT6|CLGN_BOVIN,tr|Q2E GT5|Q2EGT5_BOVIN]	In Vivo: Successfully Matured	5	4	4.96	21577.54	25076.26	454.51	280.31	47.47	9.57	1.77	6.63	0.71	0.0141	InVivo/SM>InVitro/SM
sp|Q3SZI6|RPN2_BOVIN	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 OS=Bos taurus GN=RPN2 PE=2 SV=1	In Vivo: Successfully Matured	17	17	16.91	1420655.86	541763.49	668008.57	207167.24	2.13	14.80	0.34	14.06	0.37	0.0143	InVivo/SM>InVitro/SM
tr|A0JN43|A0JN43_BOVIN	Family with sequence similarity 62 (C2 domain containing), member A OS=Bos taurus GN=FAM62A PE=2 SV=1	In Vivo: Successfully Matured	6	6	5.98	36029.41	16501.42	12471.40	5318.04	2.89	11.10	0.40	9.99	0.66	0.0145	
sp|Q3SZB7|F16P1_BOVIN	Fructose-1,6-bisphosphatase 1 OS=Bos taurus GN=FBP1 PE=2 SV=3	In Vivo: Successfully Matured	3	3	2.97	17865.05	18208.14	1293.35	1503.72	13.81	10.00	1.00	6.79	2.01	0.0146	InVivo/SM>InVitro/SM
sp|Q08DQ2|GFPT2_BOVIN	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] 2 OS=Bos taurus GN=GFPT2 PE=1 SV=1	In Vivo: Successfully Matured	5	2	4.98	8858.28	7051.90	1372.93	1362.78	6.45	9.51	0.77	7.38	1.27	0.0147	InVitro/FM>InVivo/FM
tr|A6QNS6|A6QNS6_BOVIN	NID1 protein OS=Bos taurus GN=NID1 PE=2 SV=1 [tr|A6QNS6|A6QNS6_BOVIN,tr|F 1MWN3|F1MWN3_BOVIN]	In Vivo: Successfully Matured	8	8	7.96	60245.64	71385.33	2000.47	1377.51	30.12	10.87	1.37	7.70	1.71	0.0149	InVivo/SM>InVitro/SM
tr|E1BKX7|E1BKX7_BOVIN	Uncharacterized protein OS=Bos taurus GN=FLNB PE=4 SV=2	In Vivo: Successfully Matured	12	8	11.66	35384.56	10650.16	11773.07	18577.16	3.01	11.13	0.25	9.24	1.35	0.0149	
tr|C3V9V7|C3V9V7_BOVIN	RNP24 OS=Bos taurus GN=TMED2 PE=3 SV=1	In Vivo: Successfully Matured	10	10	9.88	212434.69	67937.75	93126.70	31224.93	2.28	12.91	0.35	12.07	0.46	0.0150	
sp|P17690|APOH_BOVIN	Beta-2-glycoprotein 1 OS=Bos taurus GN=APOH PE=1 SV=4	In Vivo: Successfully Matured	4	4	3.98	54114.27	59340.84	7285.53	5645.79	7.43	11.20	0.84	9.21	1.10	0.0150	InVivo/SM>InVitro/SM; InVivo/FM>InVitro/FM
tr|E1BH79|E1BH79_BOVIN	Uncharacterized protein OS=Bos taurus GN=EF1UD1 PE=4 SV=2	In Vivo: Successfully Matured	4	4	3.82	8672.95	4331.09	2939.35	2023.12	2.95	9.65	0.49	8.51	0.63	0.0155	InVivo/SM>InVitro/SM
sp|Q17QB3|ASAH1_BOVIN	Acid ceramidase OS=Bos taurus GN=ASAH1 PE=2 SV=3	In Vivo: Successfully Matured	2	2	1.92	19671.24	15413.98	5240.76	2786.07	3.75	10.38	0.62	9.12	0.62	0.0162	
tr|G3N3D4|G3N3D4_BOVIN;tr|E1B905|E1B905_BOVIN	Uncharacterized protein OS=Bos taurus GN=KCTD12 PE=4 SV=1	In Vivo: Successfully Matured	8	8	7.80	31374.95	10997.43	14169.56	8972.48	2.21	10.99	0.36	10.13	0.50	0.0169	InVitro/SM>InVivo/SM
sp|A2VE10|CASC4_BOVIN	Protein CASC4 OS=Bos taurus GN=CASC4 PE=2 SV=1	In Vivo: Successfully Matured	5	5	4.77	110024.45	33520.85	37476.22	18316.99	2.94	12.26	0.28	11.02	0.87	0.0169	InVivo/SM>InVitro/SM
sp|P11456|MPRD_BOVIN	Cation-dependent mannose-6-phosphate receptor OS=Bos taurus GN=M6PR PE=1 SV=1	In Vivo: Successfully Matured	8	8	7.78	362929.72	72136.92	144592.49	82136.35	2.51	13.48	0.18	12.38	0.80	0.0172	
sp|A6QLZ1|HUMMR_BOVIN	Protein MGARP OS=Bos taurus GN=MGARP PE=2 SV=2	In Vivo: Successfully Matured	3	3	2.92	44406.80	24833.28	14484.87	5353.10	3.07	11.24	0.60	10.22	0.36	0.0179	
sp|P46201|UTMP_BOVIN	Uterine milk protein OS=Bos taurus PE=2 SV=1 [sp|P46201|UTMP_BOVIN,tr|C4P U73|C4PU73_BOVIN]	In Vivo: Successfully Matured	13	13	12.79	114632.87	87772.65	8767.31	4539.37	13.08	11.81	1.35	9.63	0.64	0.0180	InVitro/FM>InVivo/FM
tr|A5PJD6|A5PJD6_BOVIN	ATL3 protein OS=Bos taurus GN=ATL3 PE=2 SV=1	In Vivo: Successfully Matured	9	9	8.59	99436.20	60154.85	26666.99	14896.81	3.73	12.01	0.65	10.74	0.63	0.0183	InVivo/SM>InVitro/SM
sp|P32007|AD173_BOVIN;sp|Q2YDD9|ADT4_BOVIN;tr|F1MDK8|F1MDK8_BOVIN	ADP/ATP translocase 3 OS=Bos taurus GN=SLC25A6 PE=1 SV=3	In Vivo: Successfully Matured	26	7	25.19	806776.48	333205.04	388848.96	108412.13	2.07	14.23	0.36	13.52	0.36	0.0187	InVivo/SM>InVitro/SM
sp|Q3MHX6|OS9_BOVIN	Protein OS-9 OS=Bos taurus GN=OS9 PE=2 SV=1 [sp|Q3MHX6|OS9_BOVIN,tr|F1M X65|F1MX65_BOVIN]	In Vivo: Successfully Matured	3	3	2.98	41142.68	27847.93	8584.23	4598.02	4.79	11.13	0.60	9.46	1.08	0.0187	InVivo/SM>InVitro/SM
tr|F1MYA8|F1MYA8_BOVIN	Uncharacterized protein OS=Bos taurus GN=ATP13A1 PE=3 SV=1	In Vivo: Successfully Matured	5	5	4.95	136532.46	58036.24	33801.09	22309.99	4.04	12.43	0.44	10.76	1.18	0.0190	InVivo/SM>InVitro/SM
sp|Q08E24|SARAF_BOVIN	Store-operated calcium entry-associated regulatory factor OS=Bos taurus GN=TMEM66 PE=2 SV=1	In Vivo: Successfully Matured	6	6	5.69	28804.65	10220.65	10865.15	8462.52	2.65	10.91	0.33	9.74	0.82	0.0193	InVitro/SM>InVivo/SM
tr|B0JYK2|B0JYK2_BOVIN	NPC1 protein OS=Bos taurus GN=NPC1 PE=2 SV=1 [tr|B0JYK2|B0JYK2_BOVIN,tr|Q8G LC9|Q8GLC9_BOVIN]	In Vivo: Successfully Matured	7	7	6.92	145362.81	63357.44	48367.58	31617.29	3.01	12.51	0.37	11.23	0.89	0.0194	InVivo/SM>InVitro/SM
sp|Q3T133|TMED9_BOVIN	Transmembrane emp24 domain-containing protein 9 OS=Bos taurus GN=TMED9 PE=1 SV=1	In Vivo: Successfully Matured	7	3	6.88	553417.66	181212.44	271553.84	116935.04	2.04	13.88	0.28	13.12	0.50	0.0194	
tr|F1N405|F1N405_BOVIN	Reticulon OS=Bos taurus GN=RTN4 PE=4 SV=1 [tr|F1N405|F1N405_BOVIN,tr|Q1R M88|Q1RM88_BOVIN]	In Vivo: Successfully Matured	8	8	7.85	505711.53	96680.53	250622.00	103618.84	2.02	13.81	0.17	13.02	0.58	0.0195	InVivo/SM>InVitro/SM
tr|E1B731|E1B731_BOVIN	Uncharacterized protein OS=Bos taurus GN=TMEM165 PE=4 SV=1	In Vivo: Successfully Matured	2	2	1.99	209387.71	70107.34	29215.78	31976.14	7.17	12.90	0.29	9.83	2.36	0.0201	InVivo/SM>InVitro/SM
tr|A4IFT6|A4IFT6_BOVIN	TMED7 protein OS=Bos taurus GN=TMED7 PE=2 SV=1	In Vivo: Successfully Matured	7	7	6.83	837903.46	188311.34	416298.06	203403.73	2.01	14.31	0.18	13.51	0.59	0.0204	
sp|Q3SYT8|NCPR_BOVIN	NADPH--cytochrome P450 reductase OS=Bos taurus GN=POR PE=2 SV=3 [sp|Q3SYT8|NCPR_BOVIN,tr|A5D 9D3|A5D9D3_BOVIN]	In Vivo: Successfully Matured	34	34	33.58	1948936.73	911212.81	796737.59	348113.08	2.45	15.08	0.43	14.19	0.49	0.0204	InVivo/SM>InVitro/SM
sp|Q148N0|ODO1_BOVIN	2-oxoglutarate dehydrogenase, mitochondrial OS=Bos taurus GN=OGDH PE=2 SV=1	In Vivo: Successfully Matured	16	16	15.68	180501.31	118035.58	58425.77	21183.63	3.09	12.62	0.60	11.61	0.40	0.0208	InVitro/FM>InVivo/FM
sp|P00743|FA10_BOVIN	Coagulation factor X OS=Bos taurus GN=F10 PE=1 SV=1 [sp|P00743|FA10_BOVIN,tr|Q3MH W2|Q3MHW2_BOVIN]	In Vivo: Successfully Matured	7	7	6.95	78191.27	97095.31	3954.78	4206.07	19.77	11.01	1.46	8.55	1.02	0.0215	InVivo/SM>InVitro/SM
sp|P00257-2|ADX_BOVIN	Isoform 2 of Adrenodoxin, mitochondrial OS=Bos taurus GN=FDX1 [sp|P00257-2|ADX_BOVIN,sp|P00257|ADX_B OVIN,tr|F1N3J0|F1N3J0_BOVIN]	In Vivo: Successfully Matured	3	3	2.99	186253.95	170845.61	32559.41	16474.03	5.72	12.45	0.87	10.92	0.72	0.0220	InVivo/SM>InVitro/SM

						In Vivo: Successfully Matured		In Vivo: Failed to Mature			In Vivo: Successfully Matured		In Vivo: Failed to Mature			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
sp|P42891|ECE1_BOVIN	Endothelin-converting enzyme 1 OS=Bos taurus GN=ECE1 PE=1 SV=2	In Vivo: Successfully Matured	6	6	5.85	109035.83	63745.83	33672.05	19872.99	3.24	12.13	0.58	10.96	0.67	0.0222	InVivo/SM>InVitro/SM
tr|F1N672|F1N672_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=TM9SF2 PE=4 SV=2	In Vivo: Successfully Matured	11	11	10.72	398773.18	209787.34	122335.40	94415.86	3.26	13.45	0.56	12.16	0.82	0.0229	InVivo/SM>InVitro/SM
sp|Q3SYS6|CHP1_BOVIN	Calcineurin B homologous protein 1 OS=Bos taurus GN=CHP1 PE=2 SV=1	In Vivo: Successfully Matured	4	4	3.99	62741.08	24566.01	14778.20	10759.64	4.25	11.66	0.42	9.82	1.40	0.0232	
tr|A6H7H3|A6H7H3_BOVIN	Estradiol 17-beta-dehydrogenase 12 OS=Bos taurus GN=LOC789567 PE=2 SV=1	In Vivo: Successfully Matured	4	4	3.98	214631.30	125859.04	55530.59	32076.60	3.87	12.84	0.51	11.31	1.08	0.0239	InVivo/SM>InVitro/SM
sp|Q3ZBE9|NSDHL_BOVIN	Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating OS=Bos taurus GN=NSDHL PE=2 SV=1	In Vivo: Successfully Matured	6	6	5.97	128153.40	46849.21	51931.55	34492.59	2.47	12.40	0.32	11.34	0.77	0.0240	InVivo/SM>InVitro/SM
sp|P12624|MARCS_BOVIN	Myristoylated alanine-rich C-kinase substrate OS=Bos taurus GN=MARCKS PE=1 SV=6	In Vivo: Successfully Matured	10	10	9.67	649457.84	328993.04	209226.11	119976.97	3.10	13.94	0.58	12.78	0.67	0.0243	InVitro/FM>InVivo/FM
tr|A4FV22|A4FV22_BOVIN	TMEM87A protein OS=Bos taurus GN=TMEM87A PE=2 SV=1	In Vivo: Successfully Matured	2	2	1.90	25245.57	22031.54	5198.19	5933.52	4.86	10.51	0.82	8.74	1.11	0.0246	
sp|Q2TBI0|LBP_BOVIN	Lipopolysaccharide-binding protein OS=Bos taurus GN=LBP PE=2 SV=1 [sp|Q2TBI0|LBP_BOVIN,tr|F1MNN7|F1MNN7_BOVIN]	In Vivo: Successfully Matured	3	3	2.91	28758.70	41648.64	1888.63	1808.34	15.23	10.10	1.29	7.62	1.42	0.0249	InVivo/SM>InVitro/SM
sp|Q3ZBA6|DJB11_BOVIN	DnaJ homolog subfamily B member 11 OS=Bos taurus GN=DNAJB11 PE=2 SV=1	In Vivo: Successfully Matured	4	4	3.99	125000.84	65525.85	44729.59	20133.98	2.79	12.31	0.49	11.27	0.64	0.0251	InVivo/SM>InVitro/SM
tr|A1L539|A1L539_BOVIN	Glypican 6 OS=Bos taurus GN=GPC6 PE=2 SV=1	In Vivo: Successfully Matured	2	2	1.99	16687.94	13625.52	2393.25	3144.14	6.97	9.97	1.07	7.76	1.35	0.0259	InVivo/SM>InVitro/SM
sp|Q8SPP7|PGRP1_BOVIN	Peptidoglycan recognition protein 1 OS=Bos taurus GN=PGLYRP1 PE=1 SV=1 [sp|Q8SPP7|PGRP1_BOVIN,tr|H2CNR1|H2CNR1_BOVIN]	In Vivo: Successfully Matured	3	3	2.94	29756.45	23788.23	743.98	853.24	40.00	10.33	1.56	5.76	3.32	0.0262	InVivo/SM>InVitro/SM
tr|F1N076|F1N076_BOVIN	Uncharacterized protein OS=Bos taurus GN=CP PE=4 SV=2	In Vivo: Successfully Matured	9	9	8.80	96807.56	83824.23	3122.27	3073.33	31.01	11.86	0.82	6.91	3.97	0.0263	InVivo/SM>InVitro/SM
tr|Q0VCQ9|Q0VCQ9_BOVIN	Reticulocalbin 2, EF-hand calcium binding domain OS=Bos taurus GN=RCN2 PE=2 SV=1	In Vivo: Successfully Matured	9	9	8.88	233278.49	159924.01	53399.47	66251.22	4.37	12.82	0.74	10.96	1.28	0.0267	InVivo/SM>InVitro/SM
tr|A7E3R8|A7E3R8_BOVIN	Transmembrane protein 109 OS=Bos taurus GN=TMEM109 PE=2 SV=1 [tr|A7E3R8|A7E3R8_BOVIN,tr|Q29RH9|Q29RH9_BOVIN]	In Vivo: Successfully Matured	2	2	1.99	47405.56	22220.76	21764.88	12489.98	2.18	11.38	0.39	10.57	0.50	0.0268	InVivo/SM>InVitro/SM
sp|Q3T004|SAMP_BOVIN	Serum amyloid P-component OS=Bos taurus GN=APCS PE=2 SV=1	In Vivo: Successfully Matured	2	2	1.98	11227.80	6029.57	893.97	1226.36	12.56	9.89	0.52	5.79	3.38	0.0280	InVivo/SM>InVitro/SM
sp|P00189|CP11A_BOVIN	Cholesterol side-chain cleavage enzyme, mitochondrial OS=Bos taurus GN=CYP11A1 PE=1 SV=1 [sp|P00189|CP11A_BOVIN,tr|A3KMX9|A3KMX9_BOVIN]	In Vivo: Successfully Matured	29	29	28.44	1610768.65	1582187.37	262024.35	182861.64	6.15	14.52	0.98	12.99	0.66	0.0283	
zz|ZZ_FGCZCont0184|	gi|229552|prf||754920A albumin [Bos primigenius taurus]	In Vivo: Successfully Matured	237	1	232.92	37289.64	28328.62	4616.63	4496.96	8.08	10.90	0.87	8.20	2.05	0.0288	
sp|A7YY55|HACD3_BOVIN	Very-long-chain (3R)-3-hydroxyacyl-[acyl-carrier protein] dehydratase 3 OS=Bos taurus GN=PTPLAD1 PE=2 SV=1	In Vivo: Successfully Matured	5	5	4.97	83127.45	35579.96	35820.52	16435.95	2.32	11.95	0.36	11.05	0.65	0.0290	
tr|E1BBT8|E1BBT8_BOVIN	Uncharacterized protein OS=Bos taurus GN=EMC1 PE=4 SV=2	In Vivo: Successfully Matured	4	4	3.93	78908.46	20103.19	32277.79	16176.58	2.44	11.94	0.23	10.87	0.87	0.0290	InVivo/SM>InVitro/SM
sp|A7E3W2|LG3BP_BOVIN	Galectin-3-binding protein OS=Bos taurus GN=LGALS3BP PE=1 SV=1	In Vivo: Successfully Matured	18	18	17.70	731199.23	537830.84	228698.04	124166.32	3.20	14.00	0.61	12.88	0.66	0.0292	InVivo/SM>InVitro/SM
tr|Q0V8K9|Q0V8K9_BOVIN	Solute carrier family 29 (Nucleoside transporters), member 1 (Fragment) OS=Bos taurus GN=SLC29A1 PE=2 SV=1 [tr|Q0V8K9|Q0V8K9_BOVIN,tr|Q3ZCS3|Q3ZCS3_BOVIN]	In Vivo: Successfully Matured	4	4	3.88	240565.20	69264.11	115142.98	65469.24	2.09	13.05	0.24	12.18	0.69	0.0296	InVivo/SM>InVitro/SM
tr|A5D7G6|A5D7G6_BOVIN	ST3B protein OS=Bos taurus GN=ST3B PE=2 SV=1	In Vivo: Successfully Matured	9	9	8.78	243729.94	93573.43	105462.73	50575.68	2.31	13.04	0.32	12.11	0.70	0.0296	InVivo/SM>InVitro/SM
sp|P18246|CXA1_BOVIN	Gap junction alpha-1 protein OS=Bos taurus GN=GJA1 PE=2 SV=2	In Vivo: Successfully Matured	21	21	20.63	2671723.16	1233809.60	1030227.75	662699.02	2.59	15.42	0.36	14.29	0.87	0.0301	InVivo/SM>InVitro/SM
tr|G3MWT1|G3MWT1_BOVIN	Uncharacterized protein OS=Bos taurus GN=LOC101905477 PE=4 SV=1 [tr|G3MWT1|G3MWT1_BOVIN,tr|G3N148|G3N148_BOVIN,tr|G3N1H5|G3N1H5_BOVIN,tr|G3N1R1|G3N1R1_BOVIN]	In Vivo: Successfully Matured	2	2	1.99	35403.76	34631.02	3529.97	5176.65	10.03	10.48	1.38	8.21	1.16	0.0304	InVivo/SM>InVitro/SM
sp|Q5E9C2|DAD1_BOVIN	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD1 OS=Bos taurus GN=DAD1 PE=3 SV=3	In Vivo: Successfully Matured	4	4	3.99	400059.75	147373.22	195545.75	104954.33	2.05	13.54	0.30	12.75	0.59	0.0310	InVivo/SM>InVitro/SM
tr|F1N7T2|F1N7T2_BOVIN	Uncharacterized protein OS=Bos taurus GN=MAN2A1 PE=4 SV=2	In Vivo: Successfully Matured	3	3	2.99	57168.04	27476.27	18896.44	12238.15	3.03	11.56	0.41	10.24	1.03	0.0310	InVivo/SM>InVitro/SM
sp|Q3SZE3|LPP3_BOVIN	Lipid phosphate phosphohydrolase 3 OS=Bos taurus GN=PPAP2B PE=2 SV=1	In Vivo: Successfully Matured	3	3	2.97	15402.13	9726.34	1079.29	1307.44	14.27	10.19	0.52	6.08	3.47	0.0312	InVivo/SM>InVitro/SM
sp|Q27971|CAN2_BOVIN	Calpain-2 catalytic subunit OS=Bos taurus GN=CAPN2 PE=2 SV=2	In Vivo: Successfully Matured	12	12	11.91	225275.32	76612.05	94331.62	58008.19	2.39	12.97	0.33	11.94	0.80	0.0314	
sp|Q0VCY0|AT2A1_BOVIN	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 OS=Bos taurus GN=ATP2A1 PE=1 SV=1 [sp|Q0VCY0|AT2A1_BOVIN,tr|F1MGE7|F1MGE7_BOVIN]	In Vivo: Successfully Matured	8	2	7.81	119052.04	58518.58	48749.67	25066.58	2.44	12.28	0.45	11.35	0.62	0.0315	InVivo/SM>InVitro/SM
tr|F1N3H1|F1N3H1_BOVIN	Calumenin OS=Bos taurus GN=CALU PE=4 SV=1	In Vivo: Successfully Matured	20	20	19.77	2023661.38	631064.83	779425.45	528829.30	2.60	15.18	0.25	13.94	1.03	0.0318	
tr|F1MKS5|F1MKS5_BOVIN	Histidine-rich glycoprotein OS=Bos taurus GN=HRG PE=4 SV=2 [tr|F1MKS5|F1MKS5_BOVIN,tr|Q9BGU1|Q9BGU1_BOVIN]	In Vivo: Successfully Matured	3	1	2.95	370.76	473.73	7.91	17.69	46.87	5.05	2.70	0.87	1.95	0.0319	InVivo/SM>InVitro/SM
sp|F1N152|HTRA1_BOVIN	Serine protease HTRA1 OS=Bos taurus GN=HTRA1 PE=2 SV=1	In Vivo: Successfully Matured	8	8	7.88	16226.22	12899.87	3316.11	4390.03	4.89	10.04	0.94	8.18	1.22	0.0322	InVitro/FM>InVivo/FM
tr|F1MZ58|F1MZ58_BOVIN	Low-density lipoprotein receptor OS=Bos taurus GN=LDLR PE=4 SV=2	In Vivo: Successfully Matured	8	7	7.79	132803.02	61956.41	49851.62	26433.87	2.66	12.37	0.54	11.38	0.61	0.0323	
tr|E1B6Z6|E1B6Z6_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=LCN2 PE=3 SV=2	In Vivo: Successfully Matured	2	2	1.97	4291.69	4459.46	201.68	359.97	21.28	8.09	1.81	4.20	2.69	0.0324	InVivo/SM>InVitro/SM
tr|E1BNJ0|E1BNJ0_BOVIN	Uncharacterized protein OS=Bos taurus GN=TMEM33 PE=4 SV=2	In Vivo: Successfully Matured	2	2	1.96	16386.20	5584.28	4830.18	3976.12	3.39	10.34	0.35	8.56	1.49	0.0324	
sp|P06623|CN37_BOVIN	2',3'-cyclic-nucleotide 3'-phosphodiesterase OS=Bos taurus GN=CNP PE=2 SV=2	In Vivo: Successfully Matured	12	12	11.76	115440.30	97829.36	18151.94	14292.99	6.36	11.89	1.07	10.29	0.69	0.0328	InVivo/SM>InVitro/SM
tr|A1A3Z1|A1A3Z1_BOVIN	Low density lipoprotein receptor-related protein 8 OS=Bos taurus GN=LRP8 PE=2 SV=1	In Vivo: Successfully Matured	17	16	16.80	509851.57	511198.16	55039.79	71751.42	9.26	13.19	1.32	10.45	1.86	0.0329	InVivo/SM>InVitro/SM
tr|G3MYU9|G3MYU9_BOVIN	Uncharacterized protein OS=Bos taurus GN=NPTX2 PE=4 SV=1	In Vivo: Successfully Matured	3	3	2.95	6267.78	6379.38	546.68	294.89	11.47	8.77	1.39	6.88	0.55	0.0335	
tr|E1BMM8|E1BMM8_BOVIN	Uncharacterized protein OS=Bos taurus GN=CHPF PE=4 SV=1	In Vivo: Successfully Matured	5	5	4.97	20342.91	14897.56	5489.41	6494.59	3.71	10.38	0.70	8.71	1.25	0.0348	InVivo/SM>InVitro/SM
tr|F1N6P2|F1N6P2_BOVIN	Uncharacterized protein OS=Bos taurus GN=SURF1 PE=4 SV=2	In Vivo: Successfully Matured	9	9	8.94	151164.85	40039.18	62984.12	38312.85	2.40	12.59	0.26	11.49	0.93	0.0349	InVivo/SM>InVitro/SM
sp|Q4PJW3|CP51A_BOVIN	Lanosterol 14-alpha demethylase OS=Bos taurus GN=CYP51A1 PE=2 SV=1	In Vivo: Successfully Matured	5	5	4.95	35173.32	21977.27	10616.64	10976.11	3.31	11.00	0.58	9.39	1.26	0.0349	InVivo/SM>InVitro/SM
sp|Q2KJ53|IMPA3_BOVIN	Inositol monophosphatase 3 OS=Bos taurus GN=IMPAD1 PE=2 SV=1	In Vivo: Successfully Matured	5	5	4.80	21293.18	4766.62	7846.21	3965.09	2.71	10.64	0.20	9.37	1.10	0.0353	
sp|Q3SZ87|SSRG_BOVIN	Translocon-associated protein subunit gamma OS=Bos taurus GN=SSR3 PE=2 SV=1	In Vivo: Successfully Matured	2	2	1.94	121237.71	68226.12	53562.94	24246.72	2.26	12.29	0.46	11.49	0.50	0.0382	InVivo/SM>InVitro/SM
sp|P46194|CP19A_BOVIN	Aromatase OS=Bos taurus GN=CYP19A1 PE=2 SV=3 [sp|P46194|CP19A_BOVIN,tr|F2Z4F2|F2Z4F2_BOVIN]	In Vivo: Successfully Matured	11	11	10.90	102234.54	108977.21	15566.58	8421.58	6.57	11.69	1.07	10.22	0.58	0.0383	InVivo/SM>InVitro/SM
sp|A8YXY3|SEP15_BOVIN	15 kDa selenoprotein OS=Bos taurus GN=SEP15 PE=2 SV=2	In Vivo: Successfully Matured	2	2	1.99	27087.85	19724.54	6059.83	9708.45	4.47	10.54	0.96	8.36	1.66	0.0389	

						In Vivo: Successfully Matured		In Vivo: Failed to Mature			In Vivo: Successfully Matured		In Vivo: Failed to Mature			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|F1MNS2|F1MNS2_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=SRPK2 PE=3 SV=2	In Vivo: Successfully Matured	2	2	1.99	10448.32	4791.79	3448.20	1962.59	3.03	9.83	0.54	8.58	0.96	0.0392	InVitro/FM>InVivo/FM
tr|G8JKY2|G8JKY2_BOVIN	7-dehydrocholesterol reductase OS=Bos taurus GN=DHCR7 PE=4 SV=1	In Vivo: Successfully Matured	9	9	8.82	263203.58	143050.61	97664.27	64713.47	2.69	13.06	0.49	11.93	0.88	0.0407	InVivo/SM>InVitro/SM
tr|F1N1E5|F1N1E5_BOVIN	Thioredoxin domain-containing protein 11 OS=Bos taurus GN=TXNDC11 PE=4 SV=1	In Vivo: Successfully Matured	3	3	2.99	9013.22	17011.77	185.19	108.09	48.67	8.42	1.54	4.90	2.74	0.0414	InVivo/SM>InVitro/SM
tr|F6RM11|F6RM11_BOVIN	Uncharacterized protein OS=Bos taurus GN=NPTN PE=4 SV=1	In Vivo: Successfully Matured	3	3	2.96	107622.59	63970.49	40821.49	29102.75	2.64	12.13	0.55	11.11	0.72	0.0418	InVivo/SM>InVitro/SM
tr|E1BCV4|E1BCV4_BOVIN	Uncharacterized protein OS=Bos taurus GN=NUP98 PE=4 SV=2	In Vivo: Successfully Matured	2	2	1.98	1014.31	878.38	313.39	522.63	3.24	7.27	0.91	2.87	3.96	0.0431	
tr|Q70E76|Q70E76_BOVIN	Alpha2,3-sialyltransferase OS=Bos taurus GN=ST3GAL4 PE=2 SV=1	In Vivo: Successfully Matured	14	14	13.87	1134682.51	590881.66	473614.28	188694.16	2.40	14.52	0.50	13.66	0.57	0.0432	InVivo/SM>InVitro/SM; InVivo/FM>InVitro/FM
tr|F1MGJ5|F1MGJ5_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=SEL1L PE=4 SV=2	In Vivo: Successfully Matured	6	6	5.94	130780.08	59736.16	52976.01	27283.52	2.47	12.38	0.46	11.39	0.77	0.0440	
tr|Q24JZ4|Q24JZ4_BOVIN	Metadherin OS=Bos taurus GN=MTDH PE=2 SV=1	In Vivo: Successfully Matured	6	6	5.98	334163.33	94197.74	166062.45	84395.01	2.01	13.37	0.29	12.56	0.70	0.0458	
tr|G3X755|G3X755_BOVIN	Uncharacterized protein OS=Bos taurus GN=PLXNB2 PE=4 SV=1	In Vivo: Successfully Matured	7	7	6.74	39820.08	13970.50	17183.56	10690.32	2.32	11.24	0.31	10.19	0.93	0.0463	
sp|Q9TTJ5|RGN_BOVIN	Regucalcin OS=Bos taurus GN=RGN PE=2 SV=1	In Vivo: Successfully Matured	11	11	10.90	304696.99	304004.90	38005.97	25388.40	8.02	12.72	1.20	10.91	1.07	0.0464	
sp|Q2KJY5|PLBL2_BOVIN	Putative phospholipase B-like 2 OS=Bos taurus GN=PLBD2 PE=2 SV=1 [sp|Q2KJY5|PLBL2_BOVIN,tr|F1MIH9|F1MIH9_BOVIN]	In Vivo: Successfully Matured	11	11	10.62	97231.51	49355.43	44485.20	19008.58	2.19	12.07	0.48	11.31	0.48	0.0465	
sp|Q3SYU9|MVP_BOVIN	Major vault protein OS=Bos taurus GN=MVP PE=2 SV=1 [sp|Q3SYU9|MVP_BOVIN,tr|F1MNN6|F1MNN6_BOVIN]	In Vivo: Successfully Matured	15	15	14.72	145730.18	64743.97	71514.87	45170.59	2.04	12.51	0.38	11.72	0.62	0.0470	
tr|F1MNI5|F1MNI5_BOVIN;sp|O62664|PGH1_BOVIN	Prostaglandin G/H synthase 2 OS=Bos taurus GN=PTGS2 PE=4 SV=1	In Vivo: Successfully Matured	9	9	8.90	101594.70	134070.67	1495.55	708.70	67.93	10.50	2.17	7.87	0.64	0.0472	InVivo/SM>InVitro/SM
sp|Q05B45|T120A_BOVIN	Transmembrane protein 120A OS=Bos taurus GN=TMEM120A PE=2 SV=1	In Vivo: Successfully Matured	14	14	13.61	804164.65	451020.46	315146.82	157217.29	2.55	14.17	0.48	13.16	0.82	0.0496	InVivo/SM>InVitro/SM
tr|E1BA93|E1BA93_BOVIN	Uncharacterized protein OS=Bos taurus GN=SYNPO PE=4 SV=2	In Vivo: Failed to Mature	15	15	14.52	41165.27	15659.46	205780.16	74137.88	5.00	11.26	0.35	12.88	0.35	0.0001	InVitro/SM>InVivo/SM; InVivo/FM>InVitro/FM
sp|Q3T0E0|ATOX1_BOVIN	Copper transport protein ATOX1 OS=Bos taurus GN=ATOX1 PE=3 SV=1	In Vivo: Failed to Mature	4	4	3.91	56236.91	11556.08	224110.60	84442.98	3.99	11.61	0.21	12.95	0.39	0.0002	InVitro/SM>InVivo/SM; InVivo/FM>InVitro/FM
sp|A4FUE7|ZC21A_BOVIN	Zinc finger C2HC domain-containing protein 1A OS=Bos taurus GN=ZC2HC1A PE=2 SV=1 [sp|A4FUE7|ZC21A_BOVIN,tr|G1K1W5|G1K1W5_BOVIN]	In Vivo: Failed to Mature	2	2	1.93	1484.12	413.18	5530.92	1774.45	3.73	7.96	0.27	9.26	0.35	0.0003	InVitro/SM>InVivo/SM
tr|E1BK00|E1BK00_BOVIN;tr|E1BM39|E1BM39_BOVIN	Uncharacterized protein OS=Bos taurus GN=CUL7 PE=3 SV=2	In Vivo: Failed to Mature	2	2	1.96	1791.39	656.46	8751.64	3919.50	4.89	8.13	0.34	9.69	0.45	0.0003	
sp|Q2KIS1|RENBP_BOVIN	N-acylglucosamine 2-epimerase OS=Bos taurus GN=RENBP PE=2 SV=2	In Vivo: Failed to Mature	2	2	1.99	6303.73	2088.52	35270.15	18754.17	5.60	9.39	0.32	11.05	0.53	0.0004	InVitro/SM>InVivo/SM
sp|Q2T9V8|DTD1_BOVIN	D-tyrosyl-tRNA(Tyr) deacylase 1 OS=Bos taurus GN=DTD1 PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.99	5657.65	1811.86	14070.34	3022.59	2.49	9.29	0.28	10.23	0.22	0.0006	InVitro/SM>InVivo/SM
tr|Q3T0F9|Q3T0F9_BOVIN	Growth factor receptor-bound protein 2 OS=Bos taurus GN=GRB2 PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.97	4293.65	1338.90	14282.81	6049.16	3.33	9.02	0.29	10.19	0.40	0.0009	InVitro/SM>InVivo/SM
sp|Q6VF17|FOXL2_BOVIN	Forkhead box protein L2 OS=Bos taurus GN=FOXL2 PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.95	20635.55	7468.96	94131.64	45569.65	4.56	10.57	0.33	12.04	0.52	0.0009	InVivo/FM>InVitro/FM
tr|E1BEW6|E1BEW6_BOVIN	Uncharacterized protein OS=Bos taurus GN=EXOSC7 PE=4 SV=1	In Vivo: Failed to Mature	3	3	2.97	14870.57	3443.00	33060.21	8337.79	2.22	10.28	0.21	11.07	0.25	0.0009	
sp|Q5E9A6|VPS25_BOVIN	Vacuolar protein-sorting-associated protein 25 OS=Bos taurus GN=VPS25 PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.99	5899.28	2802.14	17706.79	2975.10	3.00	9.28	0.45	10.46	0.18	0.0011	InVitro/SM>InVivo/SM
sp|Q9TRY0|FKBP4_BOVIN	Peptidyl-prolyl cis-trans isomerase FKBP4 OS=Bos taurus GN=FKBP4 PE=1 SV=4	In Vivo: Failed to Mature	28	2	27.35	26617.06	9169.94	79212.15	28495.55	2.98	10.82	0.37	11.93	0.31	0.0013	InVitro/SM>InVivo/SM
sp|P48734|CDK1_BOVIN	Cyclin-dependent kinase 1 OS=Bos taurus GN=CDK1 PE=2 SV=2	In Vivo: Failed to Mature	4	2	3.83	3925.07	2818.78	19140.36	7498.80	4.88	8.77	0.61	10.48	0.43	0.0015	InVitro/SM>InVivo/SM
sp|Q0P5A1|DCTN3_BOVIN	Dynactin subunit 3 OS=Bos taurus GN=DCTN3 PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.98	8091.13	2068.39	21196.88	7810.30	2.62	9.66	0.23	10.60	0.36	0.0016	InVitro/SM>InVivo/SM
tr|E1BIT9|E1BIT9_BOVIN	Uncharacterized protein OS=Bos taurus GN=FUT11 PE=3 SV=1	In Vivo: Failed to Mature	2	2	1.98	3185.52	2140.59	13444.15	4743.38	4.22	8.57	0.65	10.15	0.38	0.0026	InVivo/FM>InVitro/FM
sp|Q24K16|ZADH2_BOVIN	Zinc-binding alcohol dehydrogenase domain-containing protein 2 OS=Bos taurus GN=ZADH2 PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.80	2636.57	806.82	7813.54	4389.14	2.96	8.53	0.28	9.56	0.44	0.0028	InVitro/SM>InVivo/SM
tr|Q17QL8|Q17QL8_BOVIN	Lysophospholipase II OS=Bos taurus GN=LYPLA2 PE=2 SV=1	In Vivo: Failed to Mature	4	4	3.90	57940.44	24892.10	155300.07	40577.07	2.68	11.57	0.44	12.62	0.26	0.0031	InVitro/SM>InVivo/SM
tr|F1N556|F1N556_BOVIN	Ubiquitin carboxyl-terminal hydrolase (Fragment) OS=Bos taurus GN=USP7 PE=3 SV=2 [tr|F1N556|F1N556_BOVIN,tr|M5FK76|M5FK76_BOVIN]	In Vivo: Failed to Mature	3	3	2.79	11860.39	4514.04	29564.15	9695.76	2.49	10.02	0.34	10.94	0.35	0.0039	InVitro/SM>InVivo/SM
tr|F1MT42|F1MT42_BOVIN	Follicle-stimulating hormone receptor OS=Bos taurus GN=FSHR PE=3 SV=2	In Vivo: Failed to Mature	2	2	1.98	9273.05	2457.91	24310.55	11513.97	2.62	9.80	0.25	10.71	0.43	0.0041	InVivo/SM>InVitro/SM; InVivo/FM>InVitro/FM
tr|Q2KI86|Q2KI86_BOVIN	Replication protein A2, 32kDa OS=Bos taurus GN=RPA2 PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.99	13018.43	1975.49	27566.38	9084.82	2.12	10.16	0.15	10.87	0.37	0.0043	
tr|F1MSQ6|F1MSQ6_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=NEFH PE=3 SV=2	In Vivo: Failed to Mature	5	1	4.90	30544.16	11159.45	67172.91	12015.77	2.20	10.95	0.40	11.79	0.20	0.0049	InVitro/SM>InVivo/SM
tr|F1N2X7|F1N2X7_BOVIN;tr|REV_F1N2X7|REV_F1N2X7_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=MLLT4 PE=4 SV=2	In Vivo: Failed to Mature	17	17	16.58	54260.66	23838.44	171579.99	66330.08	3.16	11.49	0.48	12.68	0.44	0.0052	InVitro/SM>InVivo/SM
tr|E1B991|E1B991_BOVIN;tr|G3MZ71|G3MZ71_BOVIN	Uncharacterized protein OS=Bos taurus GN=KRT2 PE=3 SV=2 [tr|E1B991|E1B991_BOVIN,tr|G3MZ71|G3MZ71_BOVIN]	In Vivo: Failed to Mature	27	4	25.66	9783.31	4684.52	58526.02	60498.41	5.98	9.79	0.44	11.36	0.79	0.0053	
tr|E1B8U3|E1B8U3_BOVIN	Uncharacterized protein OS=Bos taurus GN=GREB1 PE=4 SV=2	In Vivo: Failed to Mature	5	5	4.99	5938.34	3307.72	32035.98	16424.56	5.39	9.16	0.79	10.94	0.58	0.0054	InVivo/SM>InVitro/SM
sp|Q58DH8|FEN1_BOVIN	Flap endonuclease 1 OS=Bos taurus GN=FEN1 PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.87	10549.70	4605.89	34497.00	17913.70	3.27	9.85	0.49	11.05	0.46	0.0057	InVitro/SM>InVivo/SM
tr|G3MYD5|G3MYD5_BOVIN	Uncharacterized protein OS=Bos taurus GN=IVNS1ABP PE=4 SV=1	In Vivo: Failed to Mature	2	2	1.99	932.37	413.69	9312.38	9189.26	9.99	7.45	0.39	9.40	1.09	0.0061	InVitro/SM>InVivo/SM
tr|G5E6Q2|G5E6Q2_BOVIN	Uncharacterized protein OS=Bos taurus GN=ZNF358 PE=4 SV=1	In Vivo: Failed to Mature	4	4	3.95	4652.45	2605.00	27110.16	14586.51	5.83	8.96	0.67	10.71	0.78	0.0069	InVitro/SM>InVivo/SM
tr|A0JBZ9|A0JBZ9_BOVIN	Biarmipar-2 OS=Bos taurus GN=p97bcnt2 PE=2 SV=1	In Vivo: Failed to Mature	7	2	6.82	10723.42	3524.61	36215.98	25538.74	3.38	9.93	0.30	11.03	0.60	0.0070	InVitro/SM>InVivo/SM
sp|Q2NKS3|PSMG3_BOVIN	Proteasome assembly chaperone 3 OS=Bos taurus GN=PSMG3 PE=2 SV=1	In Vivo: Failed to Mature	4	4	3.96	15371.62	10611.12	43802.12	6451.73	2.85	10.11	0.71	11.37	0.15	0.0080	InVitro/SM>InVivo/SM
sp|P18203|FKB1A_BOVIN	Peptidyl-prolyl cis-trans isomerase FKBP1A OS=Bos taurus GN=FKBP1A PE=1 SV=2	In Vivo: Failed to Mature	2	2	1.99	212223.75	99399.74	484128.48	136143.60	2.28	12.86	0.45	13.75	0.26	0.0081	
tr|F1MPJ8|F1MPJ8_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=NPEPL1 PE=4 SV=2 [tr|F1MPJ8|F1MPJ8_BOVIN,tr|Q3SWX3|Q3SWX3_BOVIN]	In Vivo: Failed to Mature	2	2	1.98	1909.19	839.96	8456.54	5351.88	4.43	8.18	0.37	9.54	0.77	0.0084	InVitro/SM>InVivo/SM
sp|A6QP17|ERAP2_BOVIN	Endoplasmic reticulum aminopeptidase 2 OS=Bos taurus GN=ERAP2 PE=2 SV=1	In Vivo: Failed to Mature	4	4	3.98	2737.82	1706.75	12654.23	8195.62	4.62	8.41	0.69	9.96	0.66	0.0087	InVitro/SM>InVivo/SM
tr|A5PK76|A5PK76_BOVIN	ASPSCR1 protein OS=Bos taurus GN=ASPSCR1 PE=2 SV=1	In Vivo: Failed to Mature	4	4	3.99	7723.47	3976.96	24912.14	10382.60	3.23	9.49	0.61	10.74	0.44	0.0087	InVivo/FM>InVitro/FM
sp|Q3T0T1|PSB10_BOVIN	Proteasome subunit beta type-10 OS=Bos taurus GN=PSMB10 PE=1 SV=1	In Vivo: Failed to Mature	5	5	4.96	36119.01	12259.66	89352.98	35073.28	2.47	11.14	0.33	12.02	0.44	0.0089	InVitro/SM>InVivo/SM
sp|Q7Z7J7|FA49A_BOVIN;sp|Q2KJE3|FA49B_BOVIN	Protein FAM49A OS=Bos taurus GN=FAM49A PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.99	11560.86	8114.44	39914.63	19151.79	3.45	9.85	0.63	11.19	0.52	0.0090	InVitro/SM>InVivo/SM
tr|A6QR08|A6QR08_BOVIN	GPS1 protein OS=Bos taurus GN=GPS1 PE=2 SV=1 [tr|A6QR08|A6QR08_BOVIN,tr|F1MP10|F1MP10_BOVIN]	In Vivo: Failed to Mature	4	4	3.86	22010.21	8282.90	50997.09	16315.21	2.32	10.63	0.37	11.48	0.39	0.0100	InVitro/SM>InVivo/SM
sp|Q2KIZ8|MCM6_BOVIN	DNA replication licensing factor MCM6 OS=Bos taurus GN=MCM6 PE=2 SV=1	In Vivo: Failed to Mature	16	16	15.58	40674.52	16953.58	112861.97	46477.49	2.77	11.22	0.43	12.24	0.48	0.0102	
sp|Q56JW4|APT_BOVIN	Adenine phosphoribosyltransferase OS=Bos taurus GN=APRT PE=2 SV=1	In Vivo: Failed to Mature	5	5	4.85	73740.66	24219.87	148198.68	37952.16	2.01	11.84	0.38	12.57	0.25	0.0102	
sp|P43033|LIS1_BOVIN	Platelet-activating factor acetylhydrolase IB subunit alpha OS=Bos taurus GN=PAFAH1B1 PE=1 SV=2 [sp|P43033|LIS1_BOVIN,tr|A5D7P3|A5D7P3_BOVIN]	In Vivo: Failed to Mature	6	6	5.73	24802.53	9835.91	59236.80	20047.57	2.39	10.72	0.46	11.64	0.34	0.0105	

						In Vivo: Successfully Matured		In Vivo: Failed to Mature			In Vivo: Successfully Matured		In Vivo: Failed to Mature			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|A6QQX8|A6QQX8_BOVIN	USP39 protein OS=Bos taurus GN=USP39 PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.91	8496.72	3932.43	28863.53	16181.94	3.41	9.66	0.42	10.82	0.63	0.0107	InVitro/SM>InVivo/SM
tr|A7MBA7|A7MBA7_BOVIN	XRCC5 protein OS=Bos taurus GN=XRCC5 PE=2 SV=1 [tr|A7MBA7|A7MBA7_BOVIN,tr|G5E5D1|G5E5D1_BOVIN]	In Vivo: Failed to Mature	3	3	2.82	10694.31	4122.78	31758.89	14258.43	2.97	9.91	0.36	10.94	0.59	0.0114	
sp|P13696|PEBP1_BOVIN	Phosphatidylethanolamine-binding protein 1 OS=Bos taurus GN=PEBP1 PE=1 SV=2	In Vivo: Failed to Mature	16	16	15.82	1654006.38	545354.71	3389550.32	1065847.22	2.05	14.96	0.33	15.69	0.34	0.0117	InVitro/SM>InVivo/SM
sp|O46382|BIG1_BOVIN	Brefeldin A-inhibited guanine nucleotide-exchange protein 1 OS=Bos taurus GN=ARFGEF1 PE=1 SV=1 [sp|O46382|BIG1_BOVIN,tr|E1BP90|E1BP90_BOVIN]	In Vivo: Failed to Mature	8	8	7.73	28686.93	13580.05	80309.17	33167.54	2.80	10.84	0.52	11.91	0.46	0.0118	
tr|A0JNE9|A0JNE9_BOVIN	CTP synthase OS=Bos taurus GN=CTPS PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.94	12648.75	3041.82	41116.06	20308.69	3.25	10.11	0.22	11.17	0.69	0.0122	InVitro/SM>InVivo/SM
tr|F1MTR1|F1MTR1_BOVIN	Uncharacterized protein OS=Bos taurus GN=IQGAP2 PE=4 SV=2	In Vivo: Failed to Mature	14	13	13.70	37141.69	19493.42	116349.99	51626.86	3.13	11.06	0.61	12.27	0.49	0.0123	
tr|E1BP50|E1BP50_BOVIN	Uncharacterized protein OS=Bos taurus GN=TNKS1BP1 PE=4 SV=2	In Vivo: Failed to Mature	4	4	3.97	2870.06	1599.83	9257.66	3589.85	3.23	8.45	0.73	9.76	0.42	0.0129	InVitro/SM>InVivo/SM
sp|Q3MHE4|MSH2_BOVIN	DNA mismatch repair protein Msh2 OS=Bos taurus GN=MSH2 PE=2 SV=1	In Vivo: Failed to Mature	5	5	4.88	13252.29	5344.14	44545.14	19042.74	3.36	10.08	0.53	11.28	0.61	0.0133	InVitro/SM>InVivo/SM
tr|F6Q4T4|F6Q4T4_BOVIN	Uncharacterized protein OS=Bos taurus GN=REPS1 PE=4 SV=1	In Vivo: Failed to Mature	2	2	1.89	6728.85	2050.41	18938.24	8510.09	2.81	9.46	0.33	10.43	0.58	0.0137	InVitro/SM>InVivo/SM
tr|A5PJR3|A5PJR3_BOVIN	Dicarbonyl/L-xylulose reductase OS=Bos taurus GN=DCXR PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.92	12663.24	8130.88	42516.45	16587.91	3.36	9.88	0.83	11.29	0.38	0.0138	InVitro/SM>InVivo/SM
tr|F1MU62|F1MU62_BOVIN	Uncharacterized protein OS=Bos taurus GN=CPS17 PE=4 SV=1	In Vivo: Failed to Mature	5	5	4.94	15237.32	2873.25	34791.13	17694.81	2.28	10.31	0.16	11.05	0.49	0.0138	InVitro/SM>InVivo/SM
sp|Q3MHZ8|LEG9_BOVIN	Galectin-9 OS=Bos taurus GN=LGALS9 PE=2 SV=1 [sp|Q3MHZ8|LEG9_BOVIN,tr|F1MZT2|F1MZT2_BOVIN,tr|Q5E9E0|Q5E9E0_BOVIN]	In Vivo: Failed to Mature	3	3	2.98	2838.37	2500.10	9430.53	3524.01	3.32	8.27	0.90	9.79	0.39	0.0142	InVitro/SM>InVivo/SM
sp|Q17R14|MYO1D_BOVIN	Unconventional myosin-Id OS=Bos taurus GN=MYO1D PE=2 SV=1 [sp|Q17R14|MYO1D_BOVIN,tr|F1MJ56|F1MJ56_BOVIN]	In Vivo: Failed to Mature	4	4	3.80	9216.42	2885.06	25305.64	13842.10	2.75	9.77	0.35	10.71	0.57	0.0158	InVitro/SM>InVivo/SM
tr|A7Z055|A7Z055_BOVIN	PLAA protein OS=Bos taurus GN=PLAA PE=2 SV=1	In Vivo: Failed to Mature	8	8	7.97	24338.87	12668.26	70930.99	28155.25	2.91	10.65	0.58	11.77	0.52	0.0166	
tr|F1MBF0|F1MBF0_BOVIN	Eukaryotic translation initiation factor 2 subunit 1 OS=Bos taurus GN=EIF2A PE=4 SV=1	In Vivo: Failed to Mature	10	10	9.76	49582.85	19657.05	118084.04	42919.35	2.38	11.43	0.42	12.30	0.45	0.0169	InVitro/SM>InVivo/SM
sp|Q0III59|PDXK_BOVIN	Pyridoxal kinase OS=Bos taurus GN=PDXK PE=2 SV=1	In Vivo: Failed to Mature	12	12	11.93	58630.20	30788.44	183238.82	87147.71	3.13	11.51	0.64	12.71	0.53	0.0169	InVitro/SM>InVivo/SM; InVivo/FM>InVitro/FM
sp|Q2NL31|MTNA_BOVIN	Methylthioribose-1-phosphate isomerase OS=Bos taurus GN=MRI1 PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.98	3023.77	2194.74	12595.68	6234.91	4.17	8.36	0.97	10.01	0.57	0.0169	InVitro/SM>InVivo/SM
tr|Q0P592|Q0P592_BOVIN	Fructosamine 3 kinase related protein OS=Bos taurus GN=FN3KRP PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.88	14838.99	7625.75	38372.91	17905.02	2.59	10.18	0.49	11.16	0.48	0.0170	InVitro/SM>InVivo/SM
tr|F1N7D7|F1N7D7_BOVIN	Dystroglycan OS=Bos taurus GN=DAG1 PE=4 SV=1	In Vivo: Failed to Mature	25	25	24.83	370314.20	150459.17	766084.24	310192.36	2.07	13.45	0.38	14.19	0.36	0.0173	InVivo/FM>InVitro/FM
tr|Q17QL4|Q17QL4_BOVIN	Melanoma antigen family D, 2 OS=Bos taurus GN=MAGED2 PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.90	3319.80	3129.87	12690.29	5150.89	3.82	8.34	1.04	10.05	0.53	0.0175	InVitro/SM>InVivo/SM
sp|Q29RL9|TCEA1_BOVIN	Transcription elongation factor A protein 1 OS=Bos taurus GN=TCEA1 PE=2 SV=1 [sp|Q29RL9|TCEA1_BOVIN,tr|F1MIT2|F1MIT2_BOVIN]	In Vivo: Failed to Mature	5	5	4.98	22617.37	9499.75	66869.37	31242.58	2.96	10.60	0.56	11.70	0.54	0.0176	InVitro/SM>InVivo/SM
tr|A0JN36|A0JN36_BOVIN	RecQ protein-like (DNA helicase Q1-like) OS=Bos taurus GN=RECQL PE=2 SV=1	In Vivo: Failed to Mature	4	4	3.90	11463.97	8297.86	38265.57	21830.87	3.34	9.87	0.55	11.08	0.68	0.0181	
sp|Q8HXY9|CFDP1_BOVIN	Craniofacial development protein 1 OS=Bos taurus GN=CFDP1 PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.97	24560.34	5007.06	51611.73	17562.95	2.10	10.78	0.19	11.47	0.48	0.0183	InVitro/SM>InVivo/SM
tr|F1MXE0|F1MXE0_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=HEG1 PE=4 SV=2	In Vivo: Failed to Mature	6	6	5.73	7823.50	4283.99	35039.54	22568.69	4.48	9.48	0.65	10.92	0.83	0.0190	
sp|A1A4Q4|TMA7_BOVIN	Translation machinery-associated protein 7 OS=Bos taurus GN=TMA7 PE=3 SV=1	In Vivo: Failed to Mature	4	4	3.97	72944.08	30630.53	147839.88	48281.66	2.03	11.81	0.41	12.55	0.33	0.0191	
tr|A6QPC5|A6QPC5_BOVIN	CTNNA2 protein OS=Bos taurus GN=CTNNA2 PE=2 SV=1	In Vivo: Failed to Mature	3	2	2.86	300.22	142.78	2494.48	3163.53	8.31	6.23	0.68	7.98	1.11	0.0195	
sp|Q2KJA1|SH3G1_BOVIN;tr|Q08DX1|Q08DX1_BOVIN	Endophilin-A2 OS=Bos taurus GN=SH3GL1 PE=2 SV=1	In Vivo: Failed to Mature	6	6	5.83	17856.26	7511.77	36443.85	10486.20	2.04	10.40	0.42	11.16	0.34	0.0197	InVitro/SM>InVivo/SM
tr|E1BD88|E1BD88_BOVIN	Uncharacterized protein OS=Bos taurus GN=SPIN1 PE=4 SV=2	In Vivo: Failed to Mature	5	5	4.94	10119.45	7190.73	39998.85	19106.50	3.95	9.58	0.97	11.17	0.59	0.0202	InVivo/FM>InVitro/FM
sp|Q5E9D5|DEST_BOVIN	Destrin OS=Bos taurus GN=DSTN PE=2 SV=3	In Vivo: Failed to Mature	5	3	4.78	59854.81	31300.62	138542.58	39796.01	2.31	11.53	0.63	12.50	0.26	0.0205	InVitro/SM>InVivo/SM
sp|Q58DC5|GTPB1_BOVIN	GTP-binding protein 1 OS=Bos taurus GN=GTPBP1 PE=2 SV=2 [sp|Q58DC5|GTPB1_BOVIN,tr|A7MB33|A7MB33_BOVIN]	In Vivo: Failed to Mature	3	3	2.94	2667.61	1243.87	6878.84	3450.46	2.58	8.48	0.48	9.43	0.51	0.0206	
sp|Q5E956|TPIS_BOVIN	Triosephosphate isomerase OS=Bos taurus GN=TPI1 PE=2 SV=3	In Vivo: Failed to Mature	44	44	43.45	9147572.27	3683394.92	20257000.80	7524083.69	2.21	16.64	0.45	17.46	0.39	0.0206	
tr|A4IFE5|A4IFE5_BOVIN	LRRC8D protein OS=Bos taurus GN=LRRC8D PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.84	1276.90	988.30	6730.19	5496.23	5.27	7.53	0.85	9.21	0.92	0.0214	
tr|Q29RM7|Q29RM7_BOVIN	Fas (TNFRSF6) associated factor 1 OS=Bos taurus GN=FAF1 PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.89	8138.44	5384.41	28731.79	12415.65	3.53	9.50	0.64	10.80	0.74	0.0221	InVitro/SM>InVivo/SM
tr|A4FV09|A4FV09_BOVIN	PANK4 protein OS=Bos taurus GN=PANK4 PE=2 SV=1 [tr|A4FV09|A4FV09_BOVIN,tr|F1MLD0|F1MLD0_BOVIN]	In Vivo: Failed to Mature	2	2	1.82	771.53	483.52	3062.75	2242.84	3.97	7.20	0.53	8.47	0.82	0.0230	InVitro/SM>InVivo/SM
tr|F1MTK0|F1MTK0_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=DNAJC9 PE=4 SV=1	In Vivo: Failed to Mature	2	2	1.96	4299.97	2252.10	13259.20	6321.19	3.08	8.92	0.55	10.05	0.66	0.0233	
tr|E1BAZ4|E1BAZ4_BOVIN	Hydroxypyruvate isomerase OS=Bos taurus GN=HYI PE=3 SV=2	In Vivo: Failed to Mature	4	4	3.90	3813.92	2399.86	16732.89	9295.37	4.39	8.63	0.94	10.24	0.74	0.0236	InVitro/SM>InVivo/SM
tr|A2T1U6|A2T1U6_BOVIN	Aminopeptidase B OS=Bos taurus GN=AP-B PE=2 SV=1 [tr|A2T1U6|A2T1U6_BOVIN,tr|A4FV56|A4FV56_BOVIN]	In Vivo: Failed to Mature	4	4	3.88	32203.06	12483.03	72009.96	32094.03	2.24	10.99	0.44	11.80	0.44	0.0250	
sp|A1A4Q2|PRXD1_BOVIN	Prolyl-tRNA synthetase associated domain-containing protein 1 OS=Bos taurus GN=PRORSD1 PE=2 SV=1	In Vivo: Failed to Mature	5	5	4.85	10918.08	5804.59	38915.64	20795.55	3.56	9.74	0.87	11.13	0.60	0.0263	
tr|A5PJS4|A5PJS4_BOVIN	LOC100138178 protein (Fragment) OS=Bos taurus GN=LOC100138178 PE=2 SV=1 [tr|A5PJS4|A5PJS4_BOVIN,tr|F1N1X8|F1N1X8_BOVIN]	In Vivo: Failed to Mature	2	2	1.80	2937.94	1794.54	6404.25	2368.17	2.18	8.54	0.53	9.40	0.40	0.0265	
tr|A4FUD0|A4FUD0_BOVIN;tr|E1B884|E1B884_BOVIN	MTHFD1 protein OS=Bos taurus GN=MTHFD1 PE=2 SV=1	In Vivo: Failed to Mature	22	21	21.65	352349.60	162890.16	725517.35	249069.06	2.06	13.37	0.47	14.14	0.36	0.0268	
sp|Q5EAD2|SERA_BOVIN	D-3-phosphoglycerate dehydrogenase OS=Bos taurus GN=PHGDH PE=2 SV=3	In Vivo: Failed to Mature	19	19	18.77	591505.97	326409.05	1450863.34	578965.48	2.45	13.80	0.67	14.82	0.40	0.0283	InVitro/SM>InVivo/SM
tr|F1N1T3|F1N1T3_BOVIN	Uncharacterized protein OS=Bos taurus GN=AKAP2 PE=4 SV=2	In Vivo: Failed to Mature	20	20	19.58	209664.12	80746.22	518253.18	214597.62	2.47	12.87	0.40	13.75	0.57	0.0283	InVitro/SM>InVivo/SM
sp|Q05588|UPAR_BOVIN	Urokinase plasminogen activator surface receptor OS=Bos taurus GN=PLAUR PE=2 SV=1	In Vivo: Failed to Mature	8	8	7.87	14295.85	8009.23	30755.29	12613.94	2.15	10.13	0.50	10.96	0.40	0.0284	InVitro/SM>InVivo/SM
tr|E1BFP1|E1BFP1_BOVIN	Uncharacterized protein OS=Bos taurus GN=HEBP2 PE=4 SV=1	In Vivo: Failed to Mature	2	2	1.99	3273.63	3116.58	16867.49	9246.54	5.15	8.15	1.34	10.21	0.88	0.0292	InVitro/SM>InVivo/SM
sp|P48427|TBCA_BOVIN	Tubulin-specific chaperone A OS=Bos taurus GN=TBCA PE=1 SV=3	In Vivo: Failed to Mature	4	4	3.88	206681.08	91928.79	431271.69	159817.16	2.09	12.84	0.45	13.60	0.41	0.0301	InVivo/FM>InVitro/FM
sp|Q32KV6|SIL1_BOVIN	Nucleotide exchange factor SIL1 OS=Bos taurus GN=SIL1 PE=2 SV=1	In Vivo: Failed to Mature	12	12	11.85	169352.25	69063.47	356286.66	130704.67	2.10	12.64	0.46	13.41	0.41	0.0303	InVivo/FM>InVitro/FM
tr|F1MT41|F1MT41_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=PTGFRN PE=4 SV=1	In Vivo: Failed to Mature	28	28	27.58	1176547.06	432386.50	2429955.74	977415.09	2.07	14.61	0.38	15.32	0.45	0.0315	
sp|Q2NL01|GPX8_BOVIN	Probable glutathione peroxidase 8 OS=Bos taurus GN=GPX8 PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.76	10134.17	3029.39	24218.35	11885.84	2.39	9.88	0.28	10.66	0.60	0.0321	InVivo/FM>InVitro/FM
tr|E1BMZ9|E1BMZ9_BOVIN	Structural maintenance of chromosomes protein OS=Bos taurus GN=SMC4 PE=3 SV=2	In Vivo: Failed to Mature	3	3	2.98	2406.36	1636.13	12914.94	9885.56	5.37	8.20	0.83	9.81	1.04	0.0324	
sp|Q0VCI1|OSGEP_BOVIN	Probable tRNA N6-adenosine threonylcarbamoyltransferase OS=Bos taurus GN=OSGEP PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.97	13092.81	5374.61	33535.57	18033.51	2.56	10.10	0.40	10.98	0.62	0.0325	InVitro/SM>InVivo/SM
sp|Q08DY9|CASP3_BOVIN;sp|Q3T0P5|CASP6_BOVIN;tr|F1MB04|F1MB04_BOVIN;tr|G3X702|G3X702_BOVIN	Caspase-3 OS=Bos taurus GN=CASP3 PE=2 SV=1 [sp|Q08DY9|CASP3_BOVIN,tr|F1MB04|F1MB04_BOVIN]	In Vivo: Failed to Mature	2	2	1.98	10766.44	6265.46	28704.11	8914.81	2.67	9.71	0.89	10.92	0.33	0.0325	InVitro/SM>InVivo/SM
tr|F1MB84|F1MB84_BOVIN	Uncharacterized protein OS=Bos taurus GN=GRHPR PE=3 SV=2	In Vivo: Failed to Mature	4	4	3.99	6531.78	6022.14	22240.73	18430.97	3.41	9.15	0.81	10.48	0.71	0.0325	InVivo/FM>InVitro/FM

						In Vivo: Successfully Matured		In Vivo: Failed to Mature			In Vivo: Successfully Matured		In Vivo: Failed to Mature			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|E0AE18|E0AE18_BOVIN	Epidermal growth factor receptor OS=Bos taurus GN=EGFR PE=2 SV=1 [tr|E0AE18|E0AE18_BOVIN,tr|F1N7F3|F1N7F3_BOVIN]	In Vivo: Failed to Mature	5	5	4.95	8788.37	11829.71	21542.96	9988.18	2.45	9.20	1.00	10.59	0.46	0.0329	
tr|F1MPD1|F1MPD1_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=MRC2 PE=4 SV=1	In Vivo: Failed to Mature	14	14	13.54	103183.68	68422.09	320815.37	118708.86	3.11	11.88	1.02	13.30	0.46	0.0331	InVivo/FM>InVitro/FM
tr|F1MF35|F1MF35_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=TLL2 PE=3 SV=1	In Vivo: Failed to Mature	5	5	4.99	7003.94	3289.10	20475.58	10207.31	2.92	9.44	0.48	10.46	0.71	0.0334	
tr|Q0IIA3|Q0IIA3_BOVIN	Sorcin OS=Bos taurus GN=SRI PE=2 SV=1	In Vivo: Failed to Mature	3	3	2.82	31674.33	11038.50	66931.30	31234.74	2.11	11.00	0.33	11.71	0.49	0.0335	
sp|P80227|ACPH_BOVIN	Acylamino-acid-releasing enzyme OS=Bos taurus GN=APEH PE=1 SV=2	In Vivo: Failed to Mature	6	6	5.91	13248.21	6805.11	30003.83	11457.87	2.26	10.05	0.55	10.93	0.46	0.0344	
tr|A7YY67|A7YY67_BOVIN	ALDH1L1 protein OS=Bos taurus GN=ALDH1L1 PE=2 SV=1	In Vivo: Failed to Mature	16	16	15.76	59356.46	35580.17	291716.47	198710.77	4.91	11.39	0.94	13.00	0.96	0.0345	InVivo/FM>InVitro/FM
tr|A5D7T2|A5D7T2_BOVIN	MGC148871 protein OS=Bos taurus GN=MGC148871 PE=2 SV=1	In Vivo: Failed to Mature	6	6	5.95	12312.86	5900.71	69292.73	52782.69	5.63	9.95	0.67	11.46	1.10	0.0345	InVitro/SM>InVivo/SM
tr|A5PJZ7|A5PJZ7_BOVIN	HDAC6 protein OS=Bos taurus GN=HDAC6 PE=2 SV=1 [tr|A5PJZ7|A5PJZ7_BOVIN,tr|F1MQP3|F1MQP3_BOVIN]	In Vivo: Failed to Mature	4	4	3.87	8814.16	6376.55	35191.59	18170.56	3.99	9.23	1.35	11.05	0.53	0.0350	
sp|O97764|QOR_BOVIN	Zeta-crystallin OS=Bos taurus GN=CRYZ PE=2 SV=2	In Vivo: Failed to Mature	3	3	2.99	30777.64	12436.96	64894.47	21907.18	2.11	10.93	0.50	11.71	0.42	0.0357	
sp|Q5E9T8|KIME_BOVIN	Mevalonate kinase OS=Bos taurus GN=MVK PE=2 SV=1 [sp|Q5E9T8|KIME_BOVIN,tr|F1MC81|F1MMC8_BOVIN,tr|Q58CT1|Q58CT1_BOVIN,tr|Q5EAA2|Q5EAA2_BOVIN]	In Vivo: Failed to Mature	10	10	9.67	35000.00	15166.09	72222.36	33399.11	2.06	11.07	0.44	11.80	0.43	0.0362	InVitro/FM>InVivo/FM
tr|F1MKB7|F1MKB7_BOVIN	Uncharacterized protein OS=Bos taurus GN=GSTO1 PE=3 SV=2 [tr|F1MKB7|F1MKB7_BOVIN,tr|Q0VCE1|Q0VCE1_BOVIN]	In Vivo: Failed to Mature	3	3	2.78	6509.43	2699.34	13287.27	5672.30	2.04	9.40	0.41	10.11	0.44	0.0367	
sp|Q58DC0|CPPED_BOVIN	Calcineurin-like phosphoesterase domain-containing protein 1 OS=Bos taurus GN=CPPED1 PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.99	15675.62	5843.65	43284.53	21973.17	2.76	10.26	0.49	11.22	0.67	0.0373	InVitro/SM>InVivo/SM
sp|Q59A32|PUR2_BOVIN	Trifunctional purine biosynthetic protein adenosine-3 OS=Bos taurus GN=GART PE=2 SV=1	In Vivo: Failed to Mature	10	10	9.69	14081.18	7847.88	31839.73	12562.56	2.26	10.09	0.59	10.98	0.46	0.0382	
tr|E1B7Q0|E1B7Q0_BOVIN	Protein Hook homolog 3 OS=Bos taurus GN=HOOK3 PE=4 SV=1	In Vivo: Failed to Mature	4	4	3.83	6744.82	2788.76	15511.44	7075.93	2.30	9.43	0.42	10.23	0.57	0.0398	InVitro/SM>InVivo/SM
tr|F1MXU5|F1MXU5_BOVIN	Uncharacterized protein OS=Bos taurus GN=STARD10 PE=4 SV=2	In Vivo: Failed to Mature	3	3	2.97	771.06	849.18	3034.58	1940.82	3.94	6.82	1.06	8.45	0.91	0.0402	
sp|A4FUD9|MCM3_BOVIN	DNA replication licensing factor MCM3 OS=Bos taurus GN=MCM3 PE=2 SV=1 [sp|A4FUD9|MCM3_BOVIN,tr|G3X6V0|G3X6V0_BOVIN]	In Vivo: Failed to Mature	11	11	10.83	48571.29	25816.45	130917.29	70714.57	2.70	11.35	0.53	12.32	0.66	0.0404	
sp|P52556|BLVRB_BOVIN	Flavin reductase (NADPH) OS=Bos taurus GN=BLVRB PE=1 SV=2	In Vivo: Failed to Mature	12	12	11.94	418687.00	231791.99	884195.96	356133.18	2.11	13.50	0.56	14.32	0.42	0.0406	InVivo/FM>InVitro/FM
tr|Q3ZBE8|Q3ZBE8_BOVIN	CKLF-like MARVEL transmembrane domain containing 6 OS=Bos taurus GN=CMTM6 PE=2 SV=1	In Vivo: Failed to Mature	6	1	5.96	580.67	242.46	2165.38	1922.68	3.73	6.98	0.40	8.06	0.89	0.0417	InVitro/FM>InVivo/FM
sp|A7MB80|GTF2I_BOVIN	General transcription factor II-I OS=Bos taurus GN=GTF2I PE=2 SV=1	In Vivo: Failed to Mature	8	8	7.74	49436.86	36744.51	111452.59	50830.43	2.25	11.33	0.54	12.21	0.55	0.0439	
tr|F1MTT7|F1MTT7_BOVIN	3,2-trans-enoyl-CoA isomerase, mitochondrial OS=Bos taurus GN=ECI1 PE=4 SV=1 [tr|F1MTT7|F1MTT7_BOVIN,tr|Q2NL38|Q2NL38_BOVIN]	In Vivo: Failed to Mature	8	8	7.95	314813.20	113390.09	696278.94	370361.86	2.21	13.29	0.36	14.03	0.56	0.0444	
tr|F1MWY9|F1MWY9_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=ECI2 PE=4 SV=2	In Vivo: Failed to Mature	5	5	4.89	67459.26	24182.92	143933.92	77542.19	2.13	11.76	0.31	12.45	0.55	0.0452	
sp|Q2KIN5|HEM3_BOVIN	Porphobilinogen deaminase OS=Bos taurus GN=HMBS PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.89	4931.94	3596.34	10069.78	3791.32	2.04	8.99	0.63	9.85	0.39	0.0454	
sp|Q3SZC4|NSF1C_BOVIN	NSFL1 cofactor p47 OS=Bos taurus GN=NSFL1C PE=2 SV=1	In Vivo: Failed to Mature	16	16	15.72	406560.93	167641.14	844920.70	373309.83	2.08	13.52	0.46	14.26	0.47	0.0462	
tr|E1BLG8|E1BLG8_BOVIN	Uncharacterized protein OS=Bos taurus GN=AMPD2 PE=4 SV=2	In Vivo: Failed to Mature	4	4	3.88	7213.10	4904.46	30241.96	19083.23	4.19	9.07	1.35	10.82	0.72	0.0469	
sp|Q32L92|CNN3_BOVIN	Calponin-3 OS=Bos taurus GN=CNN3 PE=2 SV=1	In Vivo: Failed to Mature	25	25	24.71	1780420.82	1120431.48	4650934.82	1782329.30	2.61	14.78	0.93	15.97	0.46	0.0469	InVivo/FM>InVitro/FM
tr|A5PK69|A5PK69_BOVIN	RAC3 protein OS=Bos taurus GN=RAC3 PE=2 SV=1 [tr|A5PK69|A5PK69_BOVIN,tr|F1MCR0|F1MCR0_BOVIN]	In Vivo: Failed to Mature	5	2	4.95	4414.10	4851.94	23271.51	18332.14	5.27	8.49	1.14	10.32	1.21	0.0489	
tr|A4IFV2|A4IFV2_BOVIN	CA8 protein OS=Bos taurus GN=CA8 PE=2 SV=1	In Vivo: Failed to Mature	2	2	1.99	5754.54	3732.16	21827.77	15749.39	3.79	9.19	0.57	10.39	0.96	0.0490	InVivo/FM>InVitro/FM
tr|G3X696|G3X696_BOVIN	Uncharacterized protein OS=Bos taurus GN=SLK PE=4 SV=1	In Vivo: Failed to Mature	2	2	1.99	567.39	499.88	4574.43	2017.90	8.06	5.64	2.89	9.03	0.50	0.0492	InVivo/FM>InVitro/FM; InVitro/SM>InVitro/FM
tr|A5D7T6|A5D7T6_BOVIN	YY1 protein OS=Bos taurus GN=YY1 PE=2 SV=1 [tr|A5D7T6|A5D7T6_BOVIN,tr|F1MFX0|F1MFX0_BOVIN]	In Vivo: Failed to Mature	2	2	1.85	8621.68	3405.71	18273.28	6723.67	2.12	9.66	0.50	10.43	0.49	0.0496	
sp|Q58DK5|HEM2_BOVIN	Delta-aminolevulinic acid dehydratase OS=Bos taurus GN=ALAD PE=2 SV=1 [sp|Q58DK5|HEM2_BOVIN,tr|Q2KIL3|Q2KIL3_BOVIN]	In Vivo: Failed to Mature	9	9	8.74	423126.27	283355.34	1020832.47	246078.54	2.41	13.28	1.03	14.50	0.26	0.0498	

11.3 *In Vitro*: successfully matured versus failed to mature

						In Vitro: Successfully Matured		In Vitro: Failed to Mature			In Vitro: Successfully Matured		In Vitro: Failed to Mature			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
sp | Q29437 | AOCX_BOVIN	Primary amine oxidase, liver isozyme OS=Bos taurus PE=1 SV=1	In Vitro: Successfully Matured	2	2	1.96	129.84	171.98	2.38	5.32	54.53	4.72	1.50	0.63	1.42	0.0022	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM; InVivo/FM>InVitro/FM
sp | Q3SX44 | DDAH2_BOVIN	N(G),N(G)-dimethylarginine dimethylaminohydrolase 2 OS=Bos taurus GN=DDAH2 PE=2 SV=1	In Vitro: Successfully Matured	5	4	4.91	76552.05	14810.34	38180.15	13864.59	2.01	11.92	0.19	11.19	0.38	0.0049	InVitro/SM>InVivo/SM
sp | Q0VBZ9 | MRP_BOVIN;tr | G3MY11 | G3MY11_BOVIN	MARCKS-related protein OS=Bos taurus GN=MARCKSL1 PE=2 SV=1	In Vitro: Successfully Matured	6	5	5.88	369740.26	109642.68	150886.72	67046.30	2.45	13.48	0.30	12.53	0.46	0.0050	InVitro/SM>InVivo/SM
sp | Q1JPB0 | ILEU_BOVIN	Leukocyte elastase inhibitor OS=Bos taurus GN=SERPINB1 PE=2 SV=2 [sp | Q1JPB0 | ILEU_BOVIN,tr | G1K1L8 | G1K1L8_BOVIN]	In Vitro: Successfully Matured	2	1	1.99	2820.40	1429.71	573.26	552.51	4.92	8.56	0.42	6.60	1.09	0.0056	InVivo/FM>InVitro/FM
sp | Q9N2I8 | TRXR2_BOVIN;tr | F1MN10 | F1MN10_BOVIN;tr | G1K1Q2 | G1K1Q2_BOVIN;tr | G3MWU1 | G3MWU1_BOVIN	Thioredoxin reductase 2, mitochondrial OS=Bos taurus GN=TXNRD2 PE=1 SV=2 [sp | Q9N2I8 | TRXR2_BOVIN,tr | F1MN10 | F1MN10_BOVIN]	In Vitro: Successfully Matured	2	1	1.99	11158.50	4073.50	4151.02	2972.96	2.69	9.96	0.36	8.82	0.72	0.0132	
tr | Q3MHK9 | Q3MHK9_BOVIN	Fascin OS=Bos taurus GN=FSCN1 PE=2 SV=1	In Vitro: Successfully Matured	6	6	5.77	85666.06	48733.96	12995.70	11348.29	6.59	11.80	0.96	9.75	1.10	0.0139	InVitro/SM>InVivo/SM
sp | P25417 | CYTB_BOVIN;tr | A6QPZ0 | A6QPZ0_BOVIN	Cystatin-B OS=Bos taurus GN=CSTB PE=1 SV=1	In Vitro: Successfully Matured	4	4	3.81	125353.34	71860.31	46221.13	14202.08	2.71	12.30	0.57	11.39	0.33	0.0144	
sp | Q3T046 | BDH2_BOVIN	3-hydroxybutyrate dehydrogenase type 2 OS=Bos taurus GN=BDH2 PE=2 SV=1 [sp | Q3T046 | BDH2_BOVIN,tr | F1MLA4 | F1MLA4_BOVIN]	In Vitro: Successfully Matured	2	2	1.98	10152.13	4726.77	4513.21	1496.76	2.25	9.83	0.47	9.06	0.34	0.0190	InVitro/SM>InVivo/SM
tr | F1N0T1 | F1N0T1_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=2	In Vitro: Successfully Matured	4	4	3.92	3669.32	4026.81	596.83	643.77	6.15	8.50	0.99	6.46	1.49	0.0346	
tr | A6QLN6 | A6QLN6_BOVIN;tr | F1MYM9 | F1MYM9_BOVIN	MYH11 protein OS=Bos taurus GN=MYH11 PE=2 SV=1 [tr | A6QLN6 | A6QLN6_BOVIN,tr | F1MYM9 | F1MYM9_BOVIN]	In Vitro: Successfully Matured	11	1	10.59	1132.32	1015.38	333.14	664.87	3.40	7.29	1.13	2.74	3.84	0.0350	InVitro/SM>InVivo/SM
tr | G3X696 | G3X696_BOVIN	Uncharacterized protein OS=Bos taurus GN=SLK PE=4 SV=1	In Vitro: Successfully Matured	2	2	1.99	3970.06	1914.38	1791.95	3839.06	2.22	8.89	0.45	4.34	4.05	0.0371	InVivo/FM>InVivo/SM; InVivo/FM>InVitro/FM
tr | A6QLY8 | A6QLY8_BOVIN	IGFBP7 protein OS=Bos taurus GN=IGFBP7 PE=2 SV=1 [tr | A6QLY8 | A6QLY8_BOVIN,tr | F1MPP2 | F1MPP2_BOVIN]	In Vitro: Successfully Matured	2	2	1.99	3915.22	1890.09	1552.58	2098.59	2.52	8.87	0.49	7.37	1.27	0.0385	InVitro/SM>InVivo/SM
tr | F6QLF1 | F6QLF1_BOVIN;tr | F1N2J5 | F1N2J5_BOVIN	Uncharacterized protein OS=Bos taurus GN=HAPLN3 PE=4 SV=1	In Vitro: Successfully Matured	10	10	9.79	146468.51	86483.69	54359.87	43952.15	2.69	12.44	0.60	11.32	0.86	0.0429	InVivo/FM>InVitro/FM
sp | P06394 | K1C10_BOVIN;tr | A6QNZ7 | A6QNZ7_BOVIN	Keratin, type I cytoskeletal 10 OS=Bos taurus GN=KRT10 PE=3 SV=1 [sp | P06394 | K1C10_BOVIN,tr | A6QNZ7 | A6QNZ7_BOVIN]	In Vitro: Failed to Mature	44	2	42.76	358.39	354.50	16259.91	13187.71	45.37	6.08	1.19	9.91	1.31	0.0013	InVitro/FM>InVivo/FM
tr | E1B969 | E1B969_BOVIN	Uncharacterized protein OS=Bos taurus GN=DCHS1 PE=4 SV=2	In Vitro: Failed to Mature	2	2	1.85	697.33	402.62	4777.00	3389.05	6.85	7.08	0.68	8.88	0.95	0.0086	
tr | G5E513 | G5E513_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=1	In Vitro: Failed to Mature	3	2	2.97	40.60	82.71	98.97	66.20	2.44	1.86	2.71	5.13	0.63	0.0304	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM; InVitroFM>InVivoFM
tr | A6QNW7 | A6QNW7_BOVIN	CD5L protein OS=Bos taurus GN=CD5L PE=2 SV=1 [tr | A6QNW7 | A6QNW7_BOVIN,tr | F1N514 | F1N514_BOVIN]	In Vitro: Failed to Mature	7	7	6.88	3085.98	2964.05	34029.97	35960.39	11.03	8.31	1.09	10.43	1.46	0.0317	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM; InVitro/FM>InVivo/FM
sp | Q148H5 | K2C71_BOVIN	Keratin, type II cytoskeletal 71 OS=Bos taurus GN=KRT71 PE=2 SV=1	In Vitro: Failed to Mature	6	3	5.81	240.88	147.61	17426.80	28640.29	72.35	5.99	0.74	8.74	2.34	0.0362	
tr | E1BP48 | E1BP48_BOVIN	Uncharacterized protein OS=Bos taurus GN=KHDRBS2 PE=4 SV=2	In Vitro: Failed to Mature	2	1	1.90	97.24	76.04	607.19	291.35	6.24	4.36	2.37	7.00	0.51	0.0407	InVivo/SM>InVitro/SM; InVivo/FM>InVivo/SM

11.4 Not Matured: *in vitro* versus *in vivo*

						Failed to Mature: In Vivo		Failed to Mature: In Vitro			Failed to Mature: In Vivo		Failed to Mature: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|E1BSU3|E1BSU3_BOVIN	Uncharacterized protein OS=Bos taurus GN=GREB1 PE=4 SV=2	Failed to Mature: In Vivo	5	5	4.99	32035.98	16424.56	2129.52	1885.57	15.04	10.94	0.58	7.97	1.02	0.0005	InVivo/SM>InVitro/SM
sp|P48427|TBCA_BOVIN	Tubulin-specific chaperone A OS=Bos taurus GN=TBCA PE=1 SV=3	Failed to Mature: In Vivo	4	4	3.88	431271.69	159817.16	155985.85	21140.14	2.76	13.60	0.41	12.64	0.14	0.0011	InVivo/FM>InVivo/SM
sp|P38573|UPK1B_BOVIN	Uroplakin-1b OS=Bos taurus GN=UPK1B PE=1 SV=4	Failed to Mature: In Vivo	8	8	7.88	123739.74	76092.98	6577.15	5611.18	18.81	12.18	0.90	9.05	1.16	0.0014	
tr|E1BAJ4|E1BAJ4_BOVIN	Uncharacterized protein OS=Bos taurus GN=FAM47E-STBD1 PE=4 SV=1	Failed to Mature: In Vivo	17	17	16.74	984964.16	444656.32	251131.73	103543.78	3.92	14.39	0.56	13.07	0.37	0.0023	InVivo/SM>InVitro/SM
sp|Q1JPB0|ILEU_BOVIN	Leukocyte elastase inhibitor OS=Bos taurus GN=SERPINB1 PE=2 SV=2 [sp|Q1JPB0|ILEU_BOVIN,tr|G1K1L8|G1K1L8_BOVIN]	Failed to Mature: In Vivo	2	1	1.99	3803.15	2143.77	573.26	552.51	6.63	8.80	0.61	6.60	1.09	0.0043	InVitro/SM>InVitro/FM
tr|E1BC57|E1BC57_BOVIN	Tyrosine-protein kinase transmembrane receptor OS=Bos taurus GN=ROR2 PE=3 SV=1	Failed to Mature: In Vivo	8	8	7.66	148673.27	61573.70	47070.64	19874.72	3.16	12.52	0.50	11.38	0.41	0.0044	
tr|E1BA93|E1BA93_BOVIN	Uncharacterized protein OS=Bos taurus GN=SYNPO PE=4 SV=2	Failed to Mature: In Vivo	15	15	14.52	2057780.16	74137.88	99748.78	17999.74	2.06	12.88	0.35	12.19	0.18	0.0046	InVitro/SM>InVivo/SM; InVivo/FM>InVivo/SM
tr|F1N5K2|F1N5K2_BOVIN	Uncharacterized protein OS=Bos taurus GN=TTLL12 PE=4 SV=2	Failed to Mature: In Vivo	13	1	12.77	9200.60	8447.38	882.42	952.18	10.43	9.52	0.85	6.95	1.21	0.0047	
sp|Q6VFT7|FOXL2_BOVIN	Forkhead box protein L2 OS=Bos taurus GN=FOXL2 PE=2 SV=1	Failed to Mature: In Vivo	3	3	2.95	94131.64	45569.65	24828.29	13754.04	3.79	12.04	0.52	10.68	0.60	0.0049	InVivo/FM>InVivo/SM
tr|F1MYN5|F1MYN5_BOVIN	Uncharacterized protein OS=Bos taurus GN=FBLN1 PE=4 SV=2	Failed to Mature: In Vivo	19	19	18.76	630320.49	401332.39	81586.05	53004.10	7.73	13.80	0.91	11.81	0.71	0.0050	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM
sp|Q32KV6|SIL1_BOVIN	Nucleotide exchange factor SIL1 OS=Bos taurus GN=SIL1 PE=2 SV=1	Failed to Mature: In Vivo	12	12	11.85	356286.66	130704.67	147289.02	48160.11	2.42	13.41	0.41	12.55	0.32	0.0058	InVivo/FM>InVivo/SM
sp|Q3T0E0|ATOX1_BOVIN	Copper transport protein ATOX1 OS=Bos taurus GN=ATOX1 PE=3 SV=1	Failed to Mature: In Vivo	4	4	3.91	224110.60	84442.98	93556.93	32022.02	2.40	12.95	0.39	12.09	0.35	0.0063	InVitro/SM>InVivo/SM; InVivo/FM>InVivo/SM
tr|F1MB84|F1MB84_BOVIN	Uncharacterized protein OS=Bos taurus GN=GRHPR PE=3 SV=2	Failed to Mature: In Vivo	4	4	3.99	22240.73	18430.97	4757.01	2501.00	4.68	10.48	0.71	9.06	0.49	0.0063	InVivo/FM>InVivo/SM
tr|F1MRT9|F1MRT9_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=UTRN PE=4 SV=2	Failed to Mature: In Vivo	5	5	4.87	21076.04	11087.45	6244.20	2628.01	3.38	10.52	0.58	9.36	0.43	0.0068	InVivo/SM>InVitro/SM
sp|Q5E9R3|EHD1_BOVIN	EH domain-containing protein 1 OS=Bos taurus GN=EHD1 PE=1 SV=1	Failed to Mature: In Vivo	2	2	1.96	1810.02	2079.28	109.06	243.86	16.60	7.22	1.80	1.40	3.13	0.0069	
tr|F1MHQ3|F1MHQ3_BOVIN;tr|A7AY64|A7AY64_BOVIN;tr|F1N735|F1N735_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=1	Failed to Mature: In Vivo	2	2	1.80	39935.27	14664.11	17588.13	4175.25	2.27	11.22	0.43	10.45	0.23	0.0077	
tr|A7YY67|A7YY67_BOVIN	ALDH1L1 protein OS=Bos taurus GN=ALDH1L1 PE=2 SV=1	Failed to Mature: In Vivo	16	16	15.76	291716.47	198710.77	36105.91	32701.98	8.08	13.00	0.96	10.76	1.10	0.0088	InVivo/FM>InVivo/SM
tr|F1MT42|F1MT42_BOVIN	Follicle-stimulating hormone receptor OS=Bos taurus GN=FSHR PE=3 SV=2	Failed to Mature: In Vivo	2	2	1.98	24310.55	11513.97	5199.33	4900.99	4.68	10.71	0.43	8.75	1.21	0.0091	InVivo/SM>InVitro/SM; InVivo/FM>InVivo/SM
sp|Q12337|BDH_BOVIN	D-beta-hydroxybutyrate dehydrogenase, mitochondrial OS=Bos taurus GN=BDH1 PE=1 SV=2	Failed to Mature: In Vivo	2	2	1.90	1508.06	933.82	319.08	368.29	4.73	7.83	0.70	6.03	0.98	0.0104	
tr|F1N7D7|F1N7D7_BOVIN	Dystroglycan OS=Bos taurus GN=DAG1 PE=4 SV=1	Failed to Mature: In Vivo	25	25	24.83	766084.24	310192.36	344715.12	138095.77	2.22	14.19	0.36	13.38	0.42	0.0109	InVivo/FM>InVivo/SM
sp|P52556|BLVRB_BOVIN	Flavin reductase (NADPH) OS=Bos taurus GN=BLVRB PE=1 SV=2	Failed to Mature: In Vivo	12	12	11.94	884195.96	356133.18	408044.48	118637.06	2.17	14.32	0.42	13.58	0.28	0.0117	InVivo/FM>InVivo/SM
tr|A6QLW3|A6QLW3_BOVIN	RPRD1B protein OS=Bos taurus GN=RPRD1B PE=2 SV=1	Failed to Mature: In Vivo	15	15	14.62	524820.33	235167.34	192354.31	98771.39	2.73	13.77	0.52	12.77	0.45	0.0118	
tr|F6QLF1|F6QLF1_BOVIN;tr|F1N2J5|F1N2J5_BOVIN	Uncharacterized protein OS=Bos taurus GN=HAPLN3 PE=4 SV=1	Failed to Mature: In Vivo	10	10	9.79	345277.87	218689.59	54359.87	43952.15	6.35	13.17	0.97	11.32	0.86	0.0125	InVitro/SM>InVitro/FM
sp|Q29437|AOCX_BOVIN	Primary amine oxidase, liver isozyme OS=Bos taurus PE=1 SV=1	Failed to Mature: In Vivo	2	2	1.96	234.68	367.84	2.38	5.32	98.56	4.61	2.39	0.63	1.42	0.0125	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM; InVitro/SM>InVitro/FM
tr|E1BI79|E1BI79_BOVIN	Uncharacterized protein OS=Bos taurus GN=FUT11 PE=3 SV=1	Failed to Mature: In Vivo	2	2	1.98	13444.15	4743.38	4811.56	3085.51	2.79	10.15	0.38	8.97	0.75	0.0140	InVivo/FM>InVivo/SM
tr|A5PK76|A5PK76_BOVIN	ASPSCR1 protein OS=Bos taurus GN=ASPSCR1 PE=2 SV=1	Failed to Mature: In Vivo	4	4	3.99	24912.14	10382.60	11343.28	3278.28	2.20	10.74	0.44	9.99	0.32	0.0148	InVivo/FM>InVivo/SM
tr|F1N5J8|F1N5J8_BOVIN	Uncharacterized protein OS=Bos taurus GN=DECR1 PE=4 SV=1	Failed to Mature: In Vivo	5	5	4.99	160354.09	91834.87	40084.17	18690.30	4.00	12.47	0.83	11.21	0.46	0.0175	
tr|E1BD88|E1BD88_BOVIN	Uncharacterized protein OS=Bos taurus GN=SPIN1 PE=4 SV=2	Failed to Mature: In Vivo	5	5	4.94	39998.85	19106.50	12968.86	6346.97	3.08	11.17	0.59	10.04	0.61	0.0177	InVivo/FM>InVivo/SM
sp|Q32L92|CNN3_BOVIN	Calponin-3 OS=Bos taurus GN=CNN3 PE=2 SV=1	Failed to Mature: In Vivo	25	25	24.71	4650934.82	1782329.30	2293565.65	391463.27	2.03	15.97	0.46	15.33	0.17	0.0186	InVivo/FM>InVivo/SM
tr|E1BKI00|E1BKI00_BOVIN;tr|E1BM39|E1BM39_BOVIN	Uncharacterized protein OS=Bos taurus GN=CUL7 PE=3 SV=2	Failed to Mature: In Vivo	2	2	1.96	8751.64	3919.50	2997.83	1822.61	2.92	9.69	0.45	8.48	0.82	0.0204	InVivo/FM>InVivo/SM
tr|F1MF38|F1MF38_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=EMB PE=4 SV=2	Failed to Mature: In Vivo	5	5	4.90	74309.62	33377.51	19705.75	17747.66	3.77	11.82	0.51	10.10	1.24	0.0211	
sp|Q3ZBA3|PKHA2_BOVIN	Pleckstrin homology domain-containing family A member 2 OS=Bos taurus GN=PLEKHA2 PE=2 SV=1 [sp|Q3ZBA3|PKHA2_BOVIN,tr|F1MHG9|F1MHG9_BOVIN]	Failed to Mature: In Vivo	3	3	2.99	26244.02	18031.33	4913.18	5997.09	5.34	10.61	0.86	8.43	1.47	0.0212	
sp|A4IF63|TRIM2_BOVIN	Tripartite motif-containing protein 2 OS=Bos taurus GN=TRIM2 PE=2 SV=1	Failed to Mature: In Vivo	2	2	1.99	12320.65	10791.93	1797.14	1035.46	6.86	9.68	1.14	8.06	0.56	0.0216	
tr|F1MZP0|F1MZP0_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=GBE1 PE=4 SV=2	Failed to Mature: In Vivo	12	1	11.74	14167.39	11988.64	2433.82	1551.95	5.82	9.88	1.04	8.31	0.70	0.0232	
sp|O77588|PLOD1_BOVIN	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 OS=Bos taurus GN=PLOD1 PE=2 SV=2	Failed to Mature: In Vivo	35	33	34.28	1823129.67	814874.69	854948.26	357797.23	2.13	15.03	0.44	14.28	0.41	0.0234	InVivo/SM>InVitro/SM
tr|Q70E76|Q70E76_BOVIN	Alpha2,3-sialyltransferase OS=Bos taurus GN=ST3GAL4V PE=2 SV=1	Failed to Mature: In Vivo	14	14	13.87	473614.28	188694.16	155740.86	134672.14	3.04	13.66	0.57	12.32	0.91	0.0236	InVivo/SM>InVivo/FM; InVivo/SM>InVitro/SM
sp|A6BMK7|NEUR1_BOVIN	Sialidase-1 OS=Bos taurus GN=NEU1 PE=2 SV=2	Failed to Mature: In Vivo	2	2	1.97	25865.23	11390.53	6308.72	6469.20	4.10	10.70	0.73	8.88	1.28	0.0241	
tr|A4IFV2|A4IFV2_BOVIN	CA8 protein OS=Bos taurus GN=CA8 PE=2 SV=1	Failed to Mature: In Vivo	2	2	1.99	21827.77	15749.39	4442.90	2706.97	4.91	10.39	0.96	8.92	0.68	0.0241	InVivo/FM>InVivo/SM
tr|E1BHG5|E1BHG5_BOVIN	Uncharacterized protein OS=Bos taurus PE=4 SV=2	Failed to Mature: In Vivo	2	2	1.98	344821.75	158125.72	115314.42	92601.19	2.99	13.35	0.52	12.03	0.92	0.0242	InVivo/SM>InVitro/SM
tr|A2VE11|A2VE11_BOVIN	IGSF8 protein OS=Bos taurus GN=IGSF8 PE=2 SV=1 [tr|A2VE11|A2VE11_BOVIN,tr|F1MTR4|F1MTR4_BOVIN]	Failed to Mature: In Vivo	5	5	4.87	21212.00	13521.65	6315.66	6634.85	3.36	10.53	0.53	8.87	1.23	0.0244	
sp|Q2NL01|GPX8_BOVIN	Probable glutathione peroxidase 8 OS=Bos taurus GN=GPX8 PE=2 SV=1	Failed to Mature: In Vivo	3	3	2.76	24218.35	11885.84	9176.53	3810.78	2.64	10.66	0.60	9.74	0.46	0.0259	InVivo/FM>InVivo/SM
tr|Q2KIV8|Q2KIV8_BOVIN	Glutathione S-transferase mu 3 (Brain) OS=Bos taurus GN=GSTM3 PE=2 SV=1	Failed to Mature: In Vivo	25	23	24.43	1591233.50	978546.30	411461.05	78215.05	3.87	14.73	0.91	13.61	0.18	0.0278	
tr|F1N2I5|F1N2I5_BOVIN	Uncharacterized protein OS=Bos taurus GN=CMBL PE=4 SV=1	Failed to Mature: In Vivo	15	13	14.88	1749832.83	940450.86	658000.71	375943.62	2.66	14.94	0.58	13.95	0.60	0.0292	
sp|Q58CP9|STIM1_BOVIN	Stromal interaction molecule 1 OS=Bos taurus GN=STIM1 PE=2 SV=1 [sp|Q58CP9|STIM1_BOVIN,tr|B0JYL7|B0JYL7_BOVIN,tr|G3X721|G3X721_BOVIN]	Failed to Mature: In Vivo	4	4	3.96	21611.50	9921.56	7645.99	6352.58	2.83	10.55	0.60	9.38	0.79	0.0294	InVitro/SM>InVivo/SM
tr|A6QPP2|A6QPP2_BOVIN	SERPIND1 protein OS=Bos taurus GN=SERPIND1 PE=2 SV=1	Failed to Mature: In Vivo	3	3	2.98	7829.67	4966.38	2038.55	1979.43	3.84	9.43	0.81	7.93	1.02	0.0324	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM
tr|D4QBB3|D4QBB3_BOVIN	Hemoglobin beta OS=Bos taurus GN=HBB PE=3 SV=1	Failed to Mature: In Vivo	26	2	25.49	24850.30	29454.27	5537.11	4673.20	4.49	10.42	0.89	9.05	0.80	0.0331	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM
tr|G3X696|G3X696_BOVIN	Uncharacterized protein OS=Bos taurus GN=SLK PE=4 SV=1	Failed to Mature: In Vivo	2	2	1.99	4574.43	2017.90	1791.95	3839.06	2.55	9.03	0.50	4.34	4.05	0.0332	InVivo/FM>InVitro/FM; InVivo/SM>InVitro/FM
sp|A5D7L5|S39AE_BOVIN	Zinc transporter ZIP14 OS=Bos taurus GN=SLC39A14 PE=2 SV=1	Failed to Mature: In Vivo	2	2	1.99	15049.83	14682.55	1781.66	1944.25	8.45	9.71	1.37	7.62	1.24	0.0352	InVivo/SM>InVitro/SM
tr|Q2NKU1|Q2NKU1_BOVIN	Deoxyuridine triphosphatase OS=Bos taurus GN=DUT PE=2 SV=1	Failed to Mature: In Vivo	3	3	2.98	28975.39	24338.47	9354.01	3925.23	3.10	10.73	0.74	9.76	0.45	0.0366	
sp|Q0II59|PDXK_BOVIN	Pyridoxal kinase OS=Bos taurus GN=PDXK PE=2 SV=1	Failed to Mature: In Vivo	12	12	11.93	183238.82	87147.71	71704.01	47009.29	2.56	12.71	0.53	11.66	0.77	0.0374	InVitro/SM>InVivo/SM; InVivo/FM>InVivo/SM
tr|F1MKG2|F1MKG2_BOVIN	Uncharacterized protein OS=Bos taurus GN=COL6A2 PE=4 SV=2	Failed to Mature: In Vivo	2	2	1.95	9827.45	7700.18	973.16	1878.50	10.10	9.33	1.55	5.27	3.30	0.0376	InVivo/SM>InVitro/SM
sp|Q2KJH4|WDR1_BOVIN	WD repeat-containing protein 1 OS=Bos taurus GN=WDR1 PE=2 SV=3	Failed to Mature: In Vivo	14	14	13.70	302463.66	121261.76	147015.40	68169.40	2.06	13.23	0.47	12.51	0.44	0.0377	
sp|Q2KI29|TM256_BOVIN	Transmembrane protein 256 OS=Bos taurus GN=TMEM256 PE=3 SV=1	Failed to Mature: In Vivo	2	2	1.99	34037.14	22046.59	11423.07	11092.28	2.98	10.95	0.67	9.70	0.91	0.0387	
tr|K4JF16|K4JF16_BOVIN	Alpha-2-macroglobulin variant 23 OS=Bos taurus GN=A2M PE=2 SV=1	Failed to Mature: In Vivo	12	1	11.83	108.21	107.56	0.00	0.00	Infinity	3.51	3.21	0.00	0.00	0.0403	InVivo/SM>InVitro/SM
tr|F1MPD1|F1MPD1_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=MRC2 PE=4 SV=1	Failed to Mature: In Vivo	14	14	13.54	320815.37	118708.86	158309.01	71117.86	2.03	13.30	0.46	12.58	0.47	0.0413	InVivo/FM>InVivo/SM
tr|A6QPH7|A6QPH7_BOVIN	AKR7A2 protein OS=Bos taurus GN=AKR7A2 PE=2 SV=1	Failed to Mature: In Vivo	11	10	10.94	410854.60	227984.25	138250.46	48335.24	2.97	13.42	0.80	12.48	0.34	0.0415	
sp|P17690|APOH_BOVIN	Beta-2-glycoprotein 1 OS=Bos taurus GN=APOH PE=1 SV=4	Failed to Mature: In Vivo	4	4	3.98	7285.53	5645.79	755.67	1083.09	9.64	9.21	1.10	5.64	3.11	0.0422	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM
tr|F1MGU7|F1MGU7_BOVIN	Fibrinogen gamma-B chain OS=Bos taurus GN=FGG PE=4 SV=1 [tr|F1MGU7|F1MGU7_BOVIN,tr|Q3SZZ9|Q3SZZ9_BOVIN]	Failed to Mature: In Vivo	29	29	28.50	113174.18	64168.81	49746.33	32323.12	2.28	12.23	0.46	11.31	0.73	0.0443	InVivo/SM>InVitro/SM
tr|Q24K04|Q24K04_BOVIN	RAN binding protein 3 OS=Bos taurus GN=RANBP3 PE=2 SV=1	Failed to Mature: In Vivo	2	2	1.90	35076.47	33759.18	10107.89	8314.11	3.47	10.86	0.83	9.68	0.74	0.0448	

						Failed to Mature: In Vivo		Failed to Mature: In Vitro			Failed to Mature: In Vivo		Failed to Mature: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
tr|F1MZU1|F1MZU1_BOVIN;tr|F1N0C8|F1N0C8_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=LARP1 PE=4 SV=2	Failed to Mature: In Vivo	7	7	6.74	73807.52	49442.82	18636.76	15377.81	3.96	11.64	0.91	10.17	1.04	0.0450	
sp|Q3SZ19|PSMD9_BOVIN	26S proteasome non-ATPase regulatory subunit 9 OS=Bos taurus GN=PSMD9 PE=1 SV=1 [sp|Q3SZ19|PSMD9_BOVIN,tr|C3V9V8|C3V9V8_BOVIN]	Failed to Mature: In Vivo	6	6	5.98	75829.00	32972.91	36779.48	10768.72	2.06	11.83	0.53	11.17	0.33	0.0453	
sp|Q3SWY3|IMDH2_BOVIN	Inosine-5'-monophosphate dehydrogenase 2 OS=Bos taurus GN=IMPDH2 PE=2 SV=1	Failed to Mature: In Vivo	11	11	10.89	119732.54	65082.15	49478.93	32109.46	2.42	12.26	0.58	11.32	0.67	0.0476	
tr|E1BNR9|E1BNR9_BOVIN	Uncharacterized protein OS=Bos taurus GN=SEMA7A PE=4 SV=1	Failed to Mature: In Vivo	4	4	3.80	22380.18	22909.84	2110.64	1467.53	10.60	9.99	1.54	8.00	1.14	0.0486	
tr|F1MBW3|F1MBW3_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=ACSL4 PE=4 SV=1	Failed to Mature: In Vitro	19	17	18.68	19768.24	5752.18	546150.11	203553.96	27.63	10.55	0.29	13.82	0.52	0.0000	InVitro/SM>InVivo/SM; InVivo/SM>InVivo/FM
tr|A4FV05|A4FV05_BOVIN	COL18A1 protein OS=Bos taurus GN=COL18A1 PE=2 SV=1	Failed to Mature: In Vitro	8	8	7.75	1677.27	1089.22	49227.24	21078.52	29.35	7.95	0.65	11.41	0.47	0.0000	InVitro/SM>InVivo/SM
tr|G3N3D4|G3N3D4_BOVIN;tr|E1B905|E1B905_BOVIN	Uncharacterized protein OS=Bos taurus GN=KCTD12 PE=4 SV=1	Failed to Mature: In Vitro	8	8	7.80	14169.56	8972.48	225216.19	105800.69	15.89	10.13	0.50	12.91	0.54	0.0000	InVitro/SM>InVivo/SM; InVivo/SM>InVivo/FM
sp|Q5E9T8|KIME_BOVIN	Mevalonate kinase OS=Bos taurus GN=MVK PE=2 SV=1 [sp|Q5E9T8|KIME_BOVIN,tr|F1MMC8|F1MMC8_BOVIN,tr|Q58CT1|Q58CT1_BOVIN,tr|Q5EAA2|Q5EAA2_BOVIN]	Failed to Mature: In Vitro	10	10	9.67	72222.36	33399.11	337225.95	86229.97	4.67	11.80	0.43	13.40	0.24	0.0001	InVivo/FM>InVivo/SM
tr|A5D9G1|A5D9G1_BOVIN	UDP-N-acetylglucosamine pyrophosphorylase 1 OS=Bos taurus GN=UAP1 PE=2 SV=1 [tr|A5D9G1|A5D9G1_BOVIN,tr|F1MJP7|F1MJP7_BOVIN]	Failed to Mature: In Vitro	21	20	20.50	53219.88	35014.22	603981.54	278336.08	11.35	11.40	0.67	13.93	0.44	0.0001	InVitro/SM>InVivo/SM
sp|Q7YQI4|T176A_BOVIN	Transmembrane protein 176A OS=Bos taurus GN=TMEM176A PE=2 SV=1	Failed to Mature: In Vitro	4	4	3.90	2300.28	2207.50	74474.65	47086.20	32.38	8.12	0.87	11.71	0.75	0.0001	InVivo/SM>InVivo/FM
sp|P80724|BASP1_BOVIN	Brain acid soluble protein 1 OS=Bos taurus GN=BASP1 PE=1 SV=3	Failed to Mature: In Vitro	2	2	1.98	7.23	9.81	1955.39	1417.05	270.58	1.49	1.90	7.92	1.04	0.0002	InVivo/SM>InVivo/FM
sp|Q5E9F5|TAGL2_BOVIN;sp|Q3ZBY2|TAGL3_BOVIN	Transgelin-2 OS=Bos taurus GN=TAGLN2 PE=2 SV=3	Failed to Mature: In Vitro	15	15	14.89	211461.58	76547.88	876865.57	248091.46	4.15	12.90	0.39	14.34	0.32	0.0002	InVivo/FM>InVivo/SM
tr|E1BGF8|E1BGF8_BOVIN	Uncharacterized protein OS=Bos taurus GN=AGPAT9 PE=4 SV=1	Failed to Mature: In Vitro	6	6	5.95	8042.43	5357.60	125554.71	37530.61	15.61	9.36	1.06	12.40	0.28	0.0003	InVivo/SM>InVivo/FM
sp|P46193|ANXA1_BOVIN	Annexin A1 OS=Bos taurus GN=ANXA1 PE=2 SV=2 [sp|P46193|ANXA1_BOVIN,tr|F1N650|F1N650_BOVIN,tr|I6YIV1|I6YIV1_BOVIN]	Failed to Mature: In Vitro	16	16	15.67	104171.74	18894.87	495989.70	216062.76	4.76	12.23	0.17	13.72	0.51	0.0003	InVivo/FM>InVivo/SM
tr|F1N0E6|F1N0E6_BOVIN	Uncharacterized protein OS=Bos taurus GN=VWA5A PE=4 SV=2	Failed to Mature: In Vitro	20	20	19.74	12101.67	3179.14	408664.99	388342.00	33.77	10.06	0.28	13.14	1.13	0.0004	
sp|P30932|CD9_BOVIN	CD9 antigen OS=Bos taurus GN=CD9 PE=2 SV=2 [sp|P30932|CD9_BOVIN,tr|G8JKX6|G8JKX6_BOVIN]	Failed to Mature: In Vitro	4	3	3.87	11772.29	5217.15	64965.54	30248.71	5.52	9.97	0.51	11.70	0.43	0.0004	InVivo/SM>InVivo/FM
sp|P06394|K1C10_BOVIN;tr|A6QNZ7|A6QNZ7_BOVIN	Keratin, type I cytoskeletal 10 OS=Bos taurus GN=KRT10 PE=3 SV=1 [sp|P06394|K1C10_BOVIN,tr|A6QNZ7|A6QNZ7_BOVIN]	Failed to Mature: In Vitro	44	2	42.76	207.66	172.64	16259.91	13187.71	78.30	5.70	0.97	9.91	1.31	0.0004	InVitro/FM>InVitro/SM
tr|E1BJ18|E1BJ18_BOVIN	Uncharacterized protein OS=Bos taurus GN=CAPN6 PE=4 SV=1	Failed to Mature: In Vitro	16	16	15.58	7044.34	4014.38	226229.32	179721.59	32.12	9.46	0.45	12.61	1.14	0.0004	
sp|P0C0T1|ITPK1_BOVIN	Inositol-tetrakisphosphate 1-kinase OS=Bos taurus GN=ITPK1 PE=1 SV=1	Failed to Mature: In Vitro	8	8	7.92	12932.39	9266.96	162754.42	61659.29	12.59	9.85	1.01	12.62	0.44	0.0005	InVitro/SM>InVivo/SM
sp|P03969|FGF2_BOVIN	Fibroblast growth factor 2 OS=Bos taurus GN=FGF2 PE=1 SV=1	Failed to Mature: In Vitro	4	4	3.88	2328.75	2948.84	87803.80	51734.60	37.70	7.48	1.76	11.96	0.52	0.0006	InVitro/SM>InVivo/SM
sp|Q3SZI4|1433T_BOVIN;tr|REV_E1BN16|REV_E1BN16_BOVIN;tr|B0JYM5|B0JYM5_BOVIN;tr|F1MQ03|F1MQ03_BOVIN	14-3-3 protein theta OS=Bos taurus GN=YWHAQ PE=2 SV=1 [sp|Q3SZI4|1433T_BOVIN,tr|B0JYM5|B0JYM5_BOVIN]	Failed to Mature: In Vitro	35	22	34.14	1835086.10	461663.59	4374593.77	1035024.42	2.38	15.09	0.26	15.96	0.25	0.0007	
sp|Q148N0|ODO1_BOVIN	2-oxoglutarate dehydrogenase, mitochondrial OS=Bos taurus GN=OGDH PE=2 SV=1	Failed to Mature: In Vitro	16	16	15.68	58425.77	21183.63	153899.72	24117.59	2.63	11.61	0.40	12.63	0.16	0.0007	InVivo/SM>InVivo/FM
tr|G3X771|G3X771_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=ABCB1 PE=3 SV=1	Failed to Mature: In Vitro	8	6	7.82	1824.55	1434.29	16370.72	6489.60	8.97	7.87	1.01	10.33	0.40	0.0010	InVivo/SM>InVivo/FM
tr|A6H741|A6H741_BOVIN	TMEM176B protein OS=Bos taurus GN=TMEM176B PE=2 SV=1	Failed to Mature: In Vitro	3	3	2.94	5802.64	4559.13	75772.74	40998.48	13.06	9.01	1.08	11.79	0.61	0.0010	InVivo/SM>InVivo/FM
sp|Q8IE24|SARAF_BOVIN	Store-operated calcium entry-associated regulatory factor OS=Bos taurus GN=TMEM66 PE=2 SV=1	Failed to Mature: In Vitro	6	6	5.69	10865.15	8462.52	68602.67	26227.11	6.31	9.74	0.82	11.76	0.43	0.0012	InVitro/SM>InVivo/SM
sp|Q05927|5NTD_BOVIN	5'-nucleotidase OS=Bos taurus GN=N1SE PE=1 SV=2	Failed to Mature: In Vitro	19	19	18.84	38541.67	58807.55	1335095.23	1506142.10	34.64	10.57	1.15	14.22	1.22	0.0012	
sp|Q95114|MFGM_BOVIN	Lactadherin OS=Bos taurus GN=MFGE8 PE=1 SV=2 [sp|Q95114|MFGM_BOVIN,tr|F1MXX6|F1MXX6_BOVIN]	Failed to Mature: In Vitro	37	37	36.12	1012203.65	509909.87	5021030.17	2418384.83	4.96	14.41	0.54	16.02	0.51	0.0012	InVivo/SM>InVivo/FM
tr|E1BPK6|E1BPK6_BOVIN	Uncharacterized protein OS=Bos taurus GN=MYO5 PE=4 SV=2	Failed to Mature: In Vitro	18	18	17.49	95851.26	31559.16	330093.59	126143.22	3.44	12.11	0.38	13.33	0.44	0.0017	InVivo/SM>InVivo/FM
tr|A6QLZ6|A6QLZ6_BOVIN	GLRX5 protein OS=Bos taurus GN=GLRX5 PE=2 SV=1	Failed to Mature: In Vitro	8	8	7.94	246032.29	97336.47	631642.36	201021.52	2.57	13.05	0.36	14.01	0.31	0.0019	
tr|G5E513|G5E513_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus PE=4 SV=1	Failed to Mature: In Vitro	3	2	2.97	9.57	21.40	98.97	66.20	10.34	0.91	2.04	5.13	0.63	0.0022	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM; InVitro/FM>InVitro/SM
tr|A3KLR9|A3KLR9_BOVIN	Superoxide dismutase [Cu-Zn] OS=Bos taurus GN=ECSOD PE=2 SV=1	Failed to Mature: In Vitro	5	5	4.96	20993.67	8908.97	76625.82	31767.96	3.65	10.55	0.51	11.86	0.48	0.0032	
sp|Q3SYX0|NDRG1_BOVIN	Protein NDRG1 OS=Bos taurus GN=NDRG1 PE=2 SV=1	Failed to Mature: In Vitro	3	3	2.99	37845.62	14416.85	101035.73	39435.56	2.67	11.18	0.35	12.16	0.39	0.0033	InVitro/SM>InVivo/SM
sp|Q08DQ2|GFPT2_BOVIN	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] 2 OS=Bos taurus GN=GFPT2 PE=1 SV=1	Failed to Mature: In Vitro	5	2	4.98	1372.93	1362.78	24107.40	20820.26	17.56	7.38	1.27	10.37	1.11	0.0041	InVivo/SM>InVivo/FM
sp|Q28156|PDE5A_BOVIN	cGMP-specific 3',5'-cyclic phosphodiesterase OS=Bos taurus GN=PDE5A PE=1 SV=1	Failed to Mature: In Vitro	10	10	9.70	2446.18	2418.92	40252.27	40501.05	16.46	8.04	1.12	10.82	1.11	0.0042	InVivo/SM>InVivo/FM
sp|O18879|GSTA2_BOVIN	Glutathione S-transferase A2 OS=Bos taurus GN=GSTA2 PE=2 SV=4	Failed to Mature: In Vitro	29	4	28.61	7502.17	2948.99	225385.90	389721.39	30.04	9.55	0.41	12.06	1.37	0.0044	InVitro/SM>InVivo/SM
sp|Q9XSK2|CD63_BOVIN	CD63 antigen OS=Bos taurus GN=CD63 PE=2 SV=4 [sp|Q9XSK2|CD63_BOVIN,tr|B0JYM4|B0JYM4_BOVIN]	Failed to Mature: In Vitro	4	4	3.69	24335.66	4554.33	57434.17	22952.73	2.36	10.78	0.20	11.58	0.42	0.0044	InVivo/SM>InVivo/FM
tr|Q08E58|Q08E58_BOVIN	Tubulin tyrosine ligase-like family, member 12 OS=Bos taurus GN=TTLL12 PE=2 SV=1	Failed to Mature: In Vitro	13	1	12.77	315.70	475.80	10203.22	5583.15	32.32	3.26	3.71	9.82	0.51	0.0044	InVitro/SM>InVivo/SM
tr|C9EF39|C9EF39_BOVIN;tr|G9HQW1|G9HQW1_BOVIN;tr|K7QE84|K7QE84_BOVIN;tr|O46711|O46711_BOVIN;tr|Q3YJI6|Q3YJI6_BOVIN;tr|W5XHU3|W5XHU3_BOVIN	MHC class I antigen (Fragment) OS=Bos taurus GN=BoLA PE=2 SV=1	Failed to Mature: In Vitro	11	4	10.90	842.70	397.63	68117.43	112477.61	80.83	7.36	0.41	10.58	1.79	0.0044	InVitro/SM>InVivo/SM
tr|Q08DU3|Q08DU3_BOVIN	Interleukin 33 OS=Bos taurus GN=IL33 PE=2 SV=1	Failed to Mature: In Vitro	4	4	3.97	3321.40	1593.97	30532.09	37804.86	9.19	8.70	0.51	10.58	0.95	0.0047	
tr|E1BME6|E1BME6_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=HK2 PE=3 SV=2	Failed to Mature: In Vitro	33	27	32.45	518002.89	201272.04	1447619.95	533325.06	2.79	13.77	0.50	14.82	0.37	0.0051	InVitro/SM>InVivo/SM
tr|B0JYQ0|B0JYQ0_BOVIN	ALB protein OS=Bos taurus GN=ALB PE=2 SV=1	Failed to Mature: In Vitro	265	24	260.40	21075.28	7539.08	1418110.61	1382719.99	67.29	10.57	0.48	13.96	1.93	0.0052	InVivo/SM>InVivo/FM
tr|Q9GKR2|Q9GKR2_BOVIN	Uncharacterized protein OS=Bos taurus GN=VCAM1 PE=2 SV=1 [tr|Q9GKR2|Q9GKR2_BOVIN,tr|Q9GKR3|Q9GKR3_BOVIN]	Failed to Mature: In Vitro	4	4	3.87	4472.79	2575.80	20458.15	15500.72	4.57	8.95	0.63	10.45	0.62	0.0052	
tr|A6QNW7|A6QNW7_BOVIN	CD5L protein OS=Bos taurus GN=CD5L PE=2 SV=1 [tr|A6QNW7|A6QNW7_BOVIN,tr|F1N514|F1N514_BOVIN]	Failed to Mature: In Vitro	7	7	6.88	1417.78	700.27	34029.97	35960.39	24.00	7.85	0.50	10.43	1.46	0.0058	InVivo/SM>InVitro/SM; InVitro/FM>InVitro/SM; InVivo/SM>InVivo/FM
sp|Q6H236|PEG3_BOVIN	Paternally-expressed gene 3 protein OS=Bos taurus GN=PEG3 PE=2 SV=1 [sp|Q6H236|PEG3_BOVIN,tr|F1MBU3|F1MBU3_BOVIN]	Failed to Mature: In Vitro	10	10	9.81	2131.26	1873.14	53358.47	63364.70	25.04	8.01	0.95	10.85	1.43	0.0060	InVitro/SM>InVivo/SM
tr|B3VNC2|B3VNC2_BOVIN;tr|A4IFS0|A4IFS0_BOVIN	Artiodactyl-specific sub-telomeric protein OS=Bos taurus GN=LOC508098 PE=2 SV=1	Failed to Mature: In Vitro	8	8	7.90	13522.16	14791.31	226872.99	255817.65	16.78	9.61	1.30	12.49	1.25	0.0072	InVitro/SM>InVivo/SM
tr|F1N152|F1N152_BOVIN	Uncharacterized protein OS=Bos taurus GN=MAP1B PE=4 SV=2	Failed to Mature: In Vitro	31	30	30.54	64880.56	14913.07	462543.80	434037.22	7.13	11.75	0.27	13.36	0.97	0.0073	InVitro/SM>InVivo/SM
sp|Q28085|CFAH_BOVIN	Complement factor H OS=Bos taurus GN=CFH PE=1 SV=3	Failed to Mature: In Vitro	7	5	6.84	533.01	182.14	2225.03	1431.32	4.17	6.91	0.43	8.21	0.70	0.0075	InVivo/SM>InVivo/FM; InVivo/SM>InVitro/SM
tr|F1N166|F1N166_BOVIN;tr|F1N052|F1N052_BOVIN	Uncharacterized protein OS=Bos taurus GN=SMARCA1 PE=4 SV=2	Failed to Mature: In Vitro	12	8	11.65	21781.67	9636.76	81901.46	50699.18	3.76	10.58	0.52	11.86	0.61	0.0075	
tr|F1MCK2|F1MCK2_BOVIN	Uncharacterized protein OS=Bos taurus GN=AHNAK PE=4 SV=2	Failed to Mature: In Vitro	7	7	6.85	41207.46	21787.14	175741.67	86555.73	4.26	11.20	0.55	12.61	0.72	0.0081	InVivo/SM>InVivo/FM
sp|P02769|ALBU_BOVIN;zz|ZZ_FGCZCont0112|	sp|ALBU_BOVIN| [sp|P02769|ALBU_BOVIN;zz|ZZ_FGCZCont0112|]	Failed to Mature: In Vitro	290	13	284.93	524281.56	348788.29	16375909.64	16030933.45	31.23	13.67	0.71	16.50	1.74	0.0098	InVivo/FM>InVivo/FM
sp|Q05443|LUM_BOVIN	Lumican OS=Bos taurus GN=LUM PE=1 SV=1	Failed to Mature: In Vitro	3	3	2.88	4410.83	3346.46	27595.35	22249.71	6.26	8.78	0.94	10.65	0.82	0.0104	InVitro/SM>InVivo/SM
tr|F1MHG5|F1MHG5_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=ARHGAP21 PE=4 SV=2	Failed to Mature: In Vitro	2	2	1.80	233249.63	69782.63	503748.62	146235.61	2.16	13.01	0.37	13.78	0.37	0.0113	

						Failed to Mature: In Vivo		Failed to Mature: In Vitro			Failed to Mature: In Vivo		Failed to Mature: In Vitro			
Accession	Description	Highest mean condition	Peptide count	Unique peptides	Confidence score	Mean	SE	Mean	SE	Max fold change	Mean (ArcSinHyp)	SE (ArcSinHyp)	Mean (ArcSinHyp)	SE (ArcSinHyp)	Anova (p)	Other significant differences between:
sp|Q3SZ16|IAH1_BOVIN	Isoamyl acetate-hydrolyzing esterase 1 homolog OS=Bos taurus GN=IAH1 PE=2 SV=1	Failed to Mature: In Vitro	2	2	1.96	657.90	652.81	3747.21	2907.91	5.70	6.81	0.95	8.66	0.83	0.0115	
sp|P04975-2|CLCB_BOVIN	Isoform Non-brain of Clathrin light chain B OS=Bos taurus GN=CLTB	Failed to Mature: In Vitro	3	3	2.87	18726.39	6111.14	41253.61	15232.60	2.20	10.48	0.35	11.26	0.40	0.0119	
tr|E1BBS6|E1BBS6_BOVIN	Sulfhydryl oxidase (Fragment) OS=Bos taurus GN=QSOX2 PE=3 SV=2	Failed to Mature: In Vitro	6	6	5.86	75635.63	30176.33	770994.27	647626.49	10.19	11.86	0.43	13.78	1.25	0.0119	InVivo/SM>InVivo/FM
tr|F1MBN2|F1MBN2_BOVIN	Uncharacterized protein OS=Bos taurus GN=TJP2 PE=4 SV=2	Failed to Mature: In Vitro	3	3	2.93	489.66	308.58	22180.30	11606.19	45.30	5.67	3.18	10.46	0.96	0.0121	InVivo/SM>InVivo/SM
sp|P19534|CADH2_BOVIN;tr|F1MW29|F1MW29_BOVIN;tr|F1N0S5|F1N0S5_BOVIN	Cadherin-2 (Fragment) OS=Bos taurus GN=CDH2 PE=2 SV=1 [sp|P19534|CADH2_BOVIN;tr|F1N0S5|F1N0S5_BOVIN]	Failed to Mature: In Vitro	6	6	5.95	17291.09	13444.62	50459.48	18628.42	2.92	10.18	0.84	11.47	0.35	0.0132	
tr|F1N1W3|F1N1W3_BOVIN	Thrombospondin-2 OS=Bos taurus GN=THBS2 PE=4 SV=1	Failed to Mature: In Vitro	9	8	8.94	1964.34	799.61	59746.86	86423.71	30.42	8.19	0.48	10.63	1.68	0.0140	InVivo/SM>InVivo/FM
sp|Q2KJ38|F110B_BOVIN	Protein FAM110B OS=Bos taurus GN=FAM110B PE=2 SV=1 [sp|Q2KJ38|F110B_BOVIN;tr|E1BF54|E1BF54_BOVIN]	Failed to Mature: In Vitro	3	3	2.89	649.08	389.63	8728.21	9435.08	13.45	6.97	0.78	9.13	1.37	0.0153	InVivo/SM>InVivo/SM
tr|G3MX98|G3MX98_BOVIN	Uncharacterized protein OS=Bos taurus GN=KRT9 PE=4 SV=1	Failed to Mature: In Vitro	3	1	2.79	8383.26	8947.61	140135.88	128734.23	16.72	9.31	0.99	11.88	1.60	0.0156	InVivo/SM>InVivo/SM; InVivo/SM>InVivo/FM
sp|Q2KJB8|PLD3_BOVIN	Phospholipase D3 OS=Bos taurus GN=PLD3 PE=2 SV=1	Failed to Mature: In Vitro	3	3	2.99	63880.07	14666.54	140966.06	75190.17	2.21	11.73	0.27	12.46	0.46	0.0163	
tr|O77976|O77976_BOVIN;tr|Q2PS13|Q2PS13_BOVIN;tr|Q30291|Q30291_BOVIN;tr|Q3YJK4|Q3YJK4_BOVIN;tr|Q4ZJS0|Q4ZJS0_BOVIN	MHC class I antigen OS=Bos taurus GN=3349.1 PE=3 SV=2 [tr|O77976|O77976_BOVIN;tr|Q2PS13|Q2PS13_BOVIN;tr|Q30291|Q30291_BOVIN;tr|Q3YJK4|Q3YJK4_BOVIN;tr|Q4ZJS0|Q4ZJS0_BOVIN]	Failed to Mature: In Vitro	9	1	8.79	1450.26	731.40	21899.12	24132.66	15.10	7.82	0.68	9.99	1.46	0.0170	
sp|Q865V6|CAPG_BOVIN	Macrophage-capping protein OS=Bos taurus GN=CAPG PE=2 SV=1	Failed to Mature: In Vitro	5	5	4.99	3257.86	3043.88	25477.97	26359.93	7.82	8.38	1.05	10.38	1.06	0.0171	InVivo/SM>InVivo/SM
tr|F1MJH8|F1MJH8_BOVIN;tr|A6QQF8|A6QQF8_BOVIN;tr|E1BIH5|E1BIH5_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=ARHGAP18 PE=4 SV=2	Failed to Mature: In Vitro	4	4	3.88	19265.24	9725.09	39528.17	13693.93	2.05	10.46	0.49	11.23	0.32	0.0182	
sp|Q0P5J4|K1C25_BOVIN	Keratin, type I cytoskeletal 25 OS=Bos taurus GN=KRT25 PE=2 SV=1	Failed to Mature: In Vitro	11	1	10.95	1103.67	977.02	16150.17	18722.13	14.63	7.38	0.90	9.64	1.46	0.0184	InVivo/SM>InVivo/FM
sp|P46201|UTMP_BOVIN	Uterine milk protein OS=Bos taurus PE=2 SV=1 [sp|P46201|UTMP_BOVIN;tr|C4PU73|C4PU73_BOVIN]	Failed to Mature: In Vitro	13	13	12.79	8767.31	4539.37	136598.21	237240.27	15.58	9.63	0.64	11.56	1.37	0.0216	InVivo/SM>InVivo/FM
sp|P12624|MARCS_BOVIN	Myristoylated alanine-rich C-kinase substrate OS=Bos taurus GN=MARCKS PE=1 SV=6	Failed to Mature: In Vitro	10	10	9.67	209226.11	119976.97	579687.58	248588.60	2.77	12.78	0.67	13.86	0.54	0.0226	InVivo/SM>InVivo/FM
tr|F1N2D3|F1N2D3_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=TJP1 PE=4 SV=2	Failed to Mature: In Vitro	7	7	6.84	7833.89	2950.58	17278.71	7793.43	2.21	9.59	0.42	10.37	0.47	0.0246	
tr|F1N444|F1N444_BOVIN;tr|E1BL59|E1BL59_BOVIN	Uncharacterized protein OS=Bos taurus GN=LTBP1 PE=4 SV=2	Failed to Mature: In Vitro	9	9	8.66	7339.50	4161.18	60205.14	59925.21	8.20	9.24	1.23	11.25	1.07	0.0247	
sp|Q3B7M6|NEDD1_BOVIN	Protein NEDD1 OS=Bos taurus GN=NEDD1 PE=2 SV=1	Failed to Mature: In Vitro	4	4	3.79	6969.98	4167.13	21582.98	16930.51	3.10	9.37	0.68	10.49	0.62	0.0259	
sp|Q2KHU0|SERB_BOVIN	Phosphoserine phosphatase OS=Bos taurus GN=PSPH PE=2 SV=1	Failed to Mature: In Vitro	2	2	1.97	4117.11	2879.82	20944.36	19032.67	5.09	8.85	0.63	10.26	0.98	0.0263	InVitro/SM>InVivo/SM
sp|O46414|FRIH_BOVIN	Ferritin heavy chain OS=Bos taurus GN=FTH1 PE=2 SV=3 [sp|O46414|FRIH_BOVIN;tr|G1K1K0|G1K1K0_BOVIN]	Failed to Mature: In Vitro	4	4	3.89	107194.70	50996.85	266750.72	111167.21	2.49	12.14	0.66	13.10	0.48	0.0287	InVitro/SM>InVivo/SM
sp|A4IF69|NHLC2_BOVIN	NHL repeat-containing protein 2 OS=Bos taurus GN=NHLRC2 PE=2 SV=1	Failed to Mature: In Vitro	3	3	2.96	4468.23	2160.41	9348.38	3074.92	2.09	8.98	0.59	9.79	0.35	0.0290	InVitro/SM>InVivo/SM
sp|O46375|TTHY_BOVIN	Transthyretin OS=Bos taurus GN=TTR PE=1 SV=1	Failed to Mature: In Vitro	5	5	4.93	62171.93	25076.01	298620.74	255555.85	4.80	11.66	0.45	12.94	1.01	0.0317	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM
tr|E1BIR8|E1BIR8_BOVIN	Uncharacterized protein OS=Bos taurus GN=FLG2 PE=4 SV=2	Failed to Mature: In Vitro	4	4	3.98	22257.87	23176.27	90714.68	79553.45	4.08	10.24	1.14	11.84	0.80	0.0331	
sp|Q28178|TSP1_BOVIN	Thrombospondin-1 OS=Bos taurus GN=THBS1 PE=2 SV=2	Failed to Mature: In Vitro	37	2	36.42	6539.19	6422.00	55562.71	56960.12	8.50	8.80	1.59	11.08	1.20	0.0335	
zz|ZZ_FGCZCont0160|sp|Q0P5J6|K1C27_BOVIN;sp|Q148H6|K1C28_BOVIN;zz|ZZ_FGCZCont0045|	sp|K1C10_HUMAN|	Failed to Mature: In Vitro	138	29	135.60	12734170.11	7960809.55	33770417.64	21244349.51	2.65	16.92	0.55	17.87	0.62	0.0336	
tr|Q3T0N9|Q3T0N9_BOVIN	O-acyltransferase OS=Bos taurus GN=SOAT1 PE=2 SV=1	Failed to Mature: In Vitro	7	7	6.79	17330.99	19397.28	44991.01	26965.00	2.60	10.09	0.87	11.27	0.57	0.0345	
tr|F1MNS2|F1MNS2_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=SRPK2 PE=3 SV=2	Failed to Mature: In Vitro	2	2	1.99	3448.20	1962.59	16495.40	8629.71	4.78	8.58	0.96	10.15	0.99	0.0347	InVivo/SM>InVivo/FM
sp|Q28198|TPA_BOVIN	Tissue-type plasminogen activator OS=Bos taurus GN=PLAT PE=2 SV=1	Failed to Mature: In Vitro	6	6	5.96	7560.15	5343.81	31340.97	23122.04	4.15	9.31	1.01	10.80	0.84	0.0356	InVivo/SM>InVitro/SM
tr|F1MCG5|F1MCG5_BOVIN	Uncharacterized protein OS=Bos taurus GN=TAP2 PE=3 SV=1 [tr|F1MCG5|F1MCG5_BOVIN;tr|F1N2T8|F1N2T8_BOVIN;tr|Q32S33|Q32S33_BOVIN;tr|Q8SQ31|Q8SQ31_BOVIN]	Failed to Mature: In Vitro	2	2	1.91	1201.47	1095.61	2997.44	808.92	2.49	7.33	1.15	8.67	0.29	0.0357	InVitro/SM>InVivo/SM
tr|F1N3A1|F1N3A1_BOVIN	Thrombospondin-1 OS=Bos taurus GN=THBS1 PE=4 SV=1	Failed to Mature: In Vitro	37	2	36.39	21180.07	20978.47	113538.65	107707.69	5.36	10.27	0.96	11.90	1.07	0.0357	
tr|G5E5M9|G5E5M9_BOVIN	Uncharacterized protein OS=Bos taurus GN=PI15 PE=4 SV=1	Failed to Mature: In Vitro	5	4	4.99	2017.04	1313.51	11888.03	9182.21	5.89	7.84	1.42	9.76	0.96	0.0366	
sp|Q27966-2|MYO1C_BOVIN	Isoform 2 of Unconventional myosin Ic OS=Bos taurus GN=MYO1C [sp|Q27966-2|MYO1C_BOVIN;sp|Q27966-3|MYO1C_BOVIN;sp|Q27966|MYO1C_BOVIN]	Failed to Mature: In Vitro	2	2	1.91	51200.28	36246.47	1135575.83	1408563.74	22.18	11.31	0.77	13.64	1.96	0.0389	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM
tr|G3MWH1|G3MWH1_BOVIN	Uncharacterized protein OS=Bos taurus GN=TPD52L2 PE=4 SV=1 [tr|G3MWH1|G3MWH1_BOVIN;tr|Q3SYU8|Q3SYU8_BOVIN]	Failed to Mature: In Vitro	5	5	4.98	31317.72	14106.14	65088.69	25569.85	2.08	10.96	0.46	11.69	0.49	0.0406	
tr|E1BJX6|E1BJX6_BOVIN	Uncharacterized protein OS=Bos taurus GN=SCUBE3 PE=4 SV=1	Failed to Mature: In Vitro	2	2	1.99	589.69	775.51	4274.40	3630.55	7.25	5.16	3.18	8.74	0.92	0.0419	InVitro/SM>InVivo/SM
tr|A5PKH0|A5PKH0_BOVIN	TORTAIP2 protein OS=Bos taurus GN=TOR1AIP2 PE=2 SV=1	Failed to Mature: In Vitro	3	3	2.96	3687.69	2208.59	18249.62	9426.89	4.95	8.50	1.34	10.26	0.96	0.0440	InVivo/SM>InVitro/SM
tr|E1BMD4|E1BMD4_BOVIN	Uncharacterized protein OS=Bos taurus GN=PHLPP2 PE=4 SV=2	Failed to Mature: In Vitro	3	3	2.91	2887.13	1954.12	20200.60	20398.37	7.00	8.45	0.74	10.04	1.29	0.0444	
tr|A6QQ25|A6QQ25_BOVIN	Lysosomal protein transmembrane 4 beta OS=Bos taurus GN=LAPTM4B PE=2 SV=1	Failed to Mature: In Vitro	2	2	1.99	7381.43	8777.74	33682.04	22102.96	4.56	8.83	1.59	10.82	0.99	0.0446	InVivo/SM>InVivo/FM
tr|E1BDC1|E1BDC1_BOVIN	Uncharacterized protein (Fragment) OS=Bos taurus GN=GPR50 PE=3 SV=2	Failed to Mature: In Vitro	2	2	1.89	8011.65	5132.60	30456.46	30778.07	3.80	9.49	0.72	10.68	0.89	0.0486	
sp|F1N152|HTRA1_BOVIN	Serine protease HTRA1 OS=Bos taurus GN=HTRA1 PE=2 SV=1	Failed to Mature: In Vitro	8	8	7.88	3316.11	4390.03	60015.22	83019.71	18.10	8.18	1.22	10.51	1.89	0.0490	InVivo/SM>InVivo/FM
tr|Q3ZBE8|Q3ZBE8_BOVIN	CKLF-like MARVEL transmembrane domain containing 6 OS=Bos taurus GN=CMTM6 PE=2 SV=1	Failed to Mature: In Vitro	6	1	5.96	2165.38	1922.68	33673.19	42643.92	15.55	8.06	0.89	10.22	1.89	0.0492	InVivo/FM>InVivo/SM
tr|E1BN05|E1BN05_BOVIN	Uncharacterized protein OS=Bos taurus GN=GLDN PE=4 SV=2	Failed to Mature: In Vitro	3	3	2.98	210.18	254.51	1578.24	1610.23	7.51	4.78	2.23	7.47	1.35	0.0499	InVivo/SM>InVitro/SM; InVivo/SM>InVivo/FM

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13 Curriculum Vitae

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1993 – 2002	**School education** in Apples-Bière and Collège de Beausobre in Morges, Switzerland
2005	**Matura**, Gymnase de Morges, Switzerland, Biology & Chemistry
2011	**Veterinary medicine degree examination** (Master), University of Berne, Berne, Switzerland
2013 – 2019	**Preparation of the thesis** under the direction of Dr. med. vet. Jasmin Walter at the Department for Farm Animals, Clinic of Reproductive Medicine, of the Vetsuisse Faculty University of Zurich Director: Prof. Dr. med. vet. Heiner Bollwein
2013 - currently	ECAR (European College Animal Reproduction) Residency under supervision of Dr. med. vet. Jasmin Walter. Subspecialisation: ruminants
2012 – 2013	Veterinary surgeon, Châtelard small and large animals Veterinary Clinic, Bière, Switzerland
2013 – 2016	Veterinary surgeon, Clinic of Reproductive Medicine, Department of Large Animals, Vetsuisse Faculty, University of Zurich, Switzerland
2016 – 2017	Replacements in different veterinary clinics in Switzerland
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